

SOCIAL AND ACOUSTIC BEHAVIOR OF THE LEAF-ROOSTING BAT

THYROPTERA TRICOLOR

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Social and Acoustic Behavior of the Leaf-Roosting Bat *Thyroptera tricolor*

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ABSTRACT

The limited availability of refuges may represent an important factor promoting the evolution of sociality, particularly in bats. Spix's disc-winged bats (*Thyroptera tricolor*) show highly specialized morphological adaptations that enable individuals to roost inside furred musoid leaves. This roosting ecology presents major challenges, as leaves rapidly unfurl, forcing bats to locate new roosts on a daily basis. Despite the reliance of *T. tricolor* on such ephemeral roosting resources, bats form stable group associations. The purpose of this study was to characterize the behavioral patterns associated with assessing roosts and any accompanying communication strategies of Spix's disc winged bats. In the first chapter, I used video and acoustic monitoring to test the hypotheses that finding a high quality roost involves the regular assessment of leaf suitability within a group's roosting range and that acoustic signals facilitate group interactions during nightly activity. The second chapter examines the patterns of geographic variation in two contact calls regularly emitted by *T. tricolor*. I evaluate the congruence of geographic variation in the acoustic features of contact calls with genetic differentiation of two populations in Costa Rica separated by a geographic barrier. In the third chapter, using an automated telemetry system, I examined the nocturnal movement patterns of all group members within the limits of the roosting range. I determined that bats regularly monitor furred leaves within their roosting range, during which they emit a number of distinct social calls. Among the acoustic signals produced by these bats, two calls are particularly common near furred leaves. When examining the relationship between genetic distance and acoustic divergence of these two contact calls I found discordant patterns of variation, suggesting the presence of distinct modes of vocal transmission within populations. The continuous assessment of movement patterns during foraging bouts provided evidence that in general, bats remain

within close proximity of a group's roosting range and the occurrence of dyadic encounters during the course of the night. This study contributes to our understanding of the behavioral strategies used by free-ranging bats providing valuable insight into the role of shelter stability in the evolution of the *T. tricolor* social system.

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I. INTRODUCTION

Ecological Constraints on the Evolution of Communication Systems

Examining the adaptive significance of sociality has been the main focus of many theoretical and empirical studies of animal behavior (Brown 1983, Weislo 1989). While much research has focused on identifying the costs and benefits of group living, it is also important to investigate the mechanisms that directly facilitate interactions among individuals and permit group stability (Clutton-Brock 1991, Komdeur 2006). Signaling behavior is one of the most important mechanisms for enabling group associations over time (Leimar and Hammerstein 2010, Seyfarth et al. 2010). Signaling is an important component of cooperative behavior in many taxa and is used in various contexts: alarm signals (e.g. squirrels, Partan et al. 2009; treehoppers, Cocroft 1999; birds, Evans et al. 1993; nonhuman primates, Ouattara et al. 2009), parent-offspring recognition (Beecher 1982, Clutton-Brock 1991, Balcombe and McCracken 1992) and individual recognition (Sharp and Hatchwell 2005, Tibbetts and Dale 2007), among others.

Communication systems are ultimately shaped by a variety of influences. From an ecological perspective, local environmental pressures can impact long-distance communication, as many taxa use signal structures that are optimized to maximize transmission distance given the acoustic properties of a specific habitat. (i.e. Ryan et al. 1990, Wiley 1991, Brown et al. 1995, Obrist 1995, Wilczynski and Ryan 1999). The availability of limited resources can also impact the evolution of communication systems (Davies and Lundberg 1984, Andelman 1986, Travis et al. 1995, Booth-Binczik et al. 2004). Refuge habitats (i.e. nests, roosts) are especially important resources in many species, and patterns of sociality and communication can be impacted by the availability and permanence of refugia (Lanham 2001).

Behavioral function and social environment are also inextricably linked to signal structure. Marler (1955) demonstrated that alarm calls of different passerine species are strikingly similar, suggesting that selection favored the evolution of signals that are difficult to localize, since this reduces the risk of the caller being preyed upon. The pressures of colonialism and the need to discriminate among many interacting individuals can also influence signal evolution. Leonard et al. (1997) found that colonial swallows produce complex individual-specific calls, while species that live in lower group densities do not produce distinctive contact calls, and this pattern has been shown to hold across other colonial species (e.g. *penguins*: Jouventin et al. 1999; *bats*: Balcombe and McCracken 1992). Further, a positive correlation between individuality in signaling systems and social group size has been demonstrated in sciurid rodents (Pollard and Blumstein 2011) and parids (Krams et al. 2012).

Finally, the importance of evolutionary history cannot be ignored when examining correlated evolution and the adaptive function of specific traits (Harvey and Pagel 1991; Pagel 1992). In many cases, species may exhibit similar characteristics because of shared evolutionary history rather than due to similar ecological, behavioral, or social pressures. For example, Shultz et al. (2011) demonstrated that the pattern of change in sociality amongst primates has a strong phylogenetic signal, indicating that ecological factors, such as food distribution, have less of an influence on the evolution of social organization than was previously thought.

Communication Systems of Bats

Bats are an excellent system for examining how ecological, behavioral, and sociobiological factors have shaped the evolution of communication signals. Bats are highly social animals, with the vast majority of species living in groups. Yet, aspects of bat social systems vary substantially across species. Extensive variation is observed both within and

between species in group size, social structure (McCracken and Bradbury 1981; Vonhof et al. 2004; Willis and Brigham 2004; Chaverri 2010), and mating system (McCracken and Wilkinson 2000). Bats also exhibit extensive variability in ecological characteristics, which influence the evolution of sociality and associated communication systems. Roosting habitats are exceedingly diverse, especially in terms of roost permanency, ranging from permanent sites, like caves, to use of highly ephemeral structures, such as leaves (Rodriguez-Herrera et al. 2007). Roosts provide bats with protection from harsh environmental conditions, concealment from predators, and opportunities for social interactions, including mating (Kunz 1982).

With such incredible diversity in the ecology and sociality of bat species, it is not surprising that these animals exhibit complex communication systems that are designed to convey specialized information to receivers. While bats use a variety of modalities for communication, by far the most common is acoustic signaling. Compared to research on echolocation (Thomas et al. 2004), substantially fewer studies have focused on bat social calls, which are those signals that are actively exchanged between two or more individuals. In bats, social calls are key components in a variety of behaviors, including mother-pup recognition (Balcombe and McCracken 1992), mate attraction (Behr and von Helversen 2004) and advertisement of aggression (Bohn et al. 2008), or distress (Russ et al. 2004).

Social calls can also be important for mediating group formation, although little research has focused on this concept in bat systems. Female greater spear-nosed bats, *Phyllostomus hastatus*, produce group-specific screech calls when exiting a roost; these calls attract group-mates to the caller's location, which presumably facilitates group foraging (Wilkinson and Boughman 1998). White-winged vampire bats, *Diaemus youngi* (Carter et al. 2008) and pallid bats, *Antrozous pallidus* (Arnold and Wilkinson 2011) have been shown to exchange consistent,

individual-specific “contact calls” that provide information about the location of adult conspecifics, although it is not known if such calls lead to group formation. An interesting note is that all of these species roost primarily in permanent structures, such as caves; as a result, spatial memory of the roosting site may play an important role in formation of groups after periods of separation. Little is known about contact calling in bats that exploit more ephemeral resources, and as such, regularly face the challenge of locating conspecifics at a new roost.

Spix’s Disc-Winged Bats

Spix’s disk-winged bats, *Thyroptera tricolor*, use highly ephemeral roost sites (furled, tubular leaves) while still maintaining long-term, stable associations with a set of conspecifics (Vonhof et al. 2004); such associations can last for close to two years (Chaverri 2010). Chaverri et al. (2010) documented a contact calling system in which two distinct social calls are exchanged between flying and roosting bats. Flying bats actively searching for a roost produce an ‘inquiry’ call; roosting conspecifics in the area rapidly answer with a ‘response’ call, which is followed by the flying bat entering the occupied leaf roost (Figure 1.1; Chaverri and Gillam 2010; Chaverri et al. 2010). Both call types encode individual-specific signatures (Gillam and Chaverri 2012), and experimental playback studies show that in some contexts, bats preferentially respond to the calls of group members over non-group members (Chaverri et al. 2012).

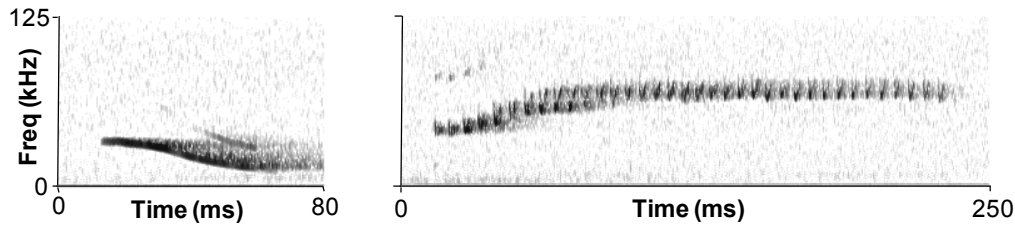


Figure 1.1. Sonograms showing a) an inquiry call and b) a response call from Spix’s disk-winged bat, *T. tricolor*

Research Objectives

The primary objective of this dissertation is to characterize the use of contact calls by *T. tricolor* and quantify the ecological, environmental, and sociobiological pressures that have impacted the evolution of this communication system. In the first chapter, I examine the behavioral patterns associated with assessing roosts and any accompanying communication strategies. I determined that bats regularly monitor furled leaves within their roosting range, during which they emit a number of distinct social calls. In the second chapter, I quantify geographic variation in microsatellite allele frequencies at multiple sites on the Caribbean and Pacific mountain slopes and test the hypothesis that patterns of acoustic differentiation align with patterns of genetic differentiation within and between regions. While I found support for this hypothesis for one type of contact call, but not for another, suggesting that these call types may have different mechanisms of transmission (social learning vs. genetic inheritance). In the third chapter, I characterize the patterns of association of *T. tricolor* in the roosting home range during the night when bats are actively flying and foraging. I discovered extensive variation in patterns of association between groups, but, overall, found that bats were consistently associated with each other (i.e. separated by only a short distance) for a significant portion of the night.

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II. BEHAVIOURAL STRATEGIES ASSOCIATED WITH USING AN EPHEMERAL ROOSTING RESOURCE IN SPIX'S DISC WINGED BAT

Introduction

Ecological factors, such as resource availability and predation pressure, can have major impacts on the evolution of social behavior in animals (Hatchwell & Komdeur, 2000; Heg et al., 2004). Such ecological constraints are a result of reliance upon resources that fluctuate with time and space, and consequently vary in availability, distribution and predictability. This variability can shape social systems in many ways and lead to outcomes ranging from solitary individuals to highly stable group associations (Wrangham et al., 1993; Chapman et al., 1995; Smith et al., 2008). Thus, examining the influence of ecological parameters on social behavior is critical for understanding the adaptive significance of sociality.

Food and shelter are among the most critical resources required by animals for effective survival and reproduction. Previous work has shown that the distribution and availability of food resources can have an important impact on patterns of group association. For instance, when food is rare or varies seasonally, group cohesion and group size decrease, mainly as a result of increased competition between conspecifics (e.g. African elephants, Wittenmyer et al., 2005; hamadryads baboons, Schreyer et al., 2012; spotted hyenas, Holtkamp et al., 2012). Less attention has been given to the role of shelters in shaping sociality, although shelters are broadly recognized as an important resource for animals. Shelters provide physical protection from predators and adverse environmental conditions (Kunz & Lumen, 2003; Kumara, 2008), and may also facilitate efficient access to food and mates (Chapman et al., 1989; Booksmythe et al., 2008). As a result, the ability of an individual to acquire a shelter will impact its survival and likelihood of reproduction.

Among bat species, the availability of roosting resources has an important influence on the strength of group associations. In turn, distinct patterns of roosting behavior have been observed in relation to the distribution of roosts in a habitat (Kerth 2008; Chaverri & Kunz 2010). For example, big brown bats, *Eptesicus fuscus*, living in buildings exhibit high roost fidelity (Brigham and Fenton 1986, Brigham 1991), while populations of the same species that roost in tree cavities regularly switch among a large number of roosts within their habitat (Kalcounis & Brigham 1998; Vonhof & Barclay 1996; Brigham et al. 1997). Despite a common pattern of low roost fidelity in many bat species, the presence of long-term associations between colony members has been observed (*Myotis bechsteinii*, Kerth & König 1999; *Eptesicus fuscus*, Willis & Brigham 2004; *Nyctalus lasiopterus*, Popa-Lisseanu et al. 2008). Roost switching behavior is even more pronounced in Spix's disc-winged bat, *Thyroptera tricolor*, compared to temperate cavity-roosting species. These bats use suction discs on the wrists and ankles to adhere to the smooth surface of developing furled leaves of *Heliconia* (Musaceae) and *Calathea* (Maranthaceae) plants that typically grow in the understory of tropical forests (Findley & Wilson 1974; Riskin & Fenton 2001). Developing leaves remain furled for up to 60h, forcing bats to switch roosts on a daily basis (Vonhof & Fenton, 2004). Despite the reliance of *T. tricolor* on such an ephemeral roosting resource, bats form long-lasting stable social groups composed primarily of close kin (Chaverri 2010; Buchalski et al., 2014). The specialized roosting ecology of *T. tricolor*, coupled with the cohesive social structure observed in roosting groups, suggest the presence of behavioral strategies that enable individuals to regroup after periods of separation. Previous experiments have identified two social calls that are involved in reuniting group mates when separated (Chaverri et al., 2010; Gillam & Chaverri 2012). However, aside from the recent studies documenting the use of these two calls, we know little about the communication system

of *T. tricolor*. Further, nothing is known about the behavioral processes involved in how a group selects a new roost – a process that invariably happens every day.

The objective of the current study is to characterize the behavioral strategies involved in locating and selecting new roosts. We used continuous video and acoustic monitoring to test the hypotheses that finding a suitable roost involves individuals: i) assessing the suitability of furled leaves within their roosting range, and ii) exchanging social calls in a manner that facilitates coordination with group members during or after foraging bouts. While we initially assumed that any occupied leaf would remain so until the next evening (i.e., a standard day roost), we found a large number of leaves were used exclusively as night roosts. As a result, our post-hoc analyses focus on trying to understand how patterns of behavior and social call production differ near night roosts, day roosts, and unoccupied leaves. Studying free-ranging bats allowed us to gain a better understanding of how individuals select new roosts and the potential benefits of social cohesion in bats that rely on highly ephemeral resources.

Methods

Study locations

The study was conducted from January to February 2011 and from March to April 2012 at three protected areas in Costa Rica. This period corresponds to the driest months at these sites which coincide with the breeding season of *T. tricolor*. Fieldwork during 2011 was carried out at Sirena field station (SIR, 8.48° N, -83.59° W), located in the south Pacific coast within Corcovado National Park. Fieldwork in 2012 was conducted at two sites in the northeast Atlantic region: 1) Cuatro Esquinas field station at Tortuguero National Park (TOR, 10.54° N, -83.50° W), located in the northeastern coast, and Tapiria National Reserve (TAP, 10.53° N, -84.03° W), located in the foothills of the Cordillera Central mountain range. The study sites are

characterized by the presence of primary and secondary tropical rainforest, with numerous streams draining throughout the study area. Plants used by *T. tricolor* as roosts are typically distributed along streams and forest gaps; however the composition and abundance of these plants differed between our study sites; the study area at Sirena exhibited dense patches of *Calathea* and *Heliconia* spp., whereas at Tortuguero, patches of *Calathea* spp. were dominant, and at Tapiria, both *Heliconia* and *Calathea* spp. were highly scattered and usually not found in dense stands (BKM, personal observation).

Capture and marking techniques

During the day, we searched for bats roosting in furled *Heliconia* spp. and *Calathea* spp. leaves. Bats were captured by pinching the top of the leaf and directing individuals into a cloth holding bag. We recorded standard measurements (body mass, forearm length, sex, reproductive state and age) of all the individuals captured. Individual identification and group membership was assessed using two different methods. Bats at Sirena field site were marked with passive integrated transponders (9 mm PIT tags; Biomark Inc., Idaho) that were implanted subcutaneously between the scapulae (tags represented <2 % of the average weight of bats). Individuals at Tortuguero and Tapiria were marked with colored forearm rings (males and females were marked in the right and left forearm respectively) (Avinet, Inc., Dryden NY). At these two sites, we also attached reflective tape to the dorsal pelage, which enabled us to recognize the individuals of a focal group monitored during the night. Reflective tape fell off after approximately 5 days (BKM, personal observation). Only adults and sub-adults were marked during the study. Measurements and marking of a focal group lasted no more than 30 minutes. Bats were released simultaneously, which typically allowed individuals to regroup in close proximity to the previous day roost.

Recording methods

At each site we selected three to seven independent groups and monitored the approach behavior of bats to known and potential furled leaf roosts using video and acoustic recordings. Focal groups were not selected at random; rather we selected groups for which we had identified at least three roosting leaves and for which the estimated roosting range was greater than 50 m apart. This enabled us to obtain a preliminary estimate of the boundaries in which a group will roost (by means of minimum convex polygons based on georeferenced locations) and reduce the possibility of recording leaves in areas where adjacent groups exhibit high spatial overlap. Day roosts were identified by positioning a mirror attached to an extending 2 m pole above an uncoiled leaf or by means of a PIT tag portable reader (PocketReaderEx, Biomark). Once the focal group was located, we placed a video and acoustic recording devices in front of the day roost if we predicted that the leaf would remain furled during the night. If the leaf would not remain furled, the recording devices were placed in front of a previously-unoccupied suitable leaf. The recording set-up was the same for both previous roosts and suitable leaves. The following criteria were used to select the suitable leaf to be monitored on a given night: i) we searched for suitable leaves with structural characteristics that roughly matched previous work describing roost dimensions (Vonhof & Fenton, 2004) and our own observations of occupied leaves in our field sites (5-7 cm diameter opening, 140-220 cm total height), ii) we identified multiple furled leaves within the estimated roosting range and we selected the closest leaf to the roost in which bats were found.

The recording system consisted of a night-shot camera (Sony HDR-CX550V) enclosed in a water resistant case attached to an infrared light source (Wildlife Engineering, IRLamp6) and a microphone ultrasonic recording system (Avisoft Bioacoustics). The video camera and lighting

system were aimed at the distal end of the leaf, positioned about 3 m away from the focal leaf. The camera was zoomed in to obtain a viewing radius around the leaf entrance of approximately 2 m. A microphone (Avisoft condenser microphone CM16) was positioned at a distance of roughly 30 cm, and was oriented towards the leaf entrance to capture acoustic signals emitted by bats approaching, entering or exiting the roost. Video recordings started before dusk (1750 h) and were continued until daylight (0530 h). These times were selected based on previous field observations in which bats would depart and enter a roost respectively. Acoustic recording at Sirena field station were conducted in 2 sessions: from 1750h to 2100h and from 0330h to 0530h. Acoustic recordings in Tortuguero and Tapiria were conducted continuously during the night. Due to the sensitivity of the microphones, we interrupted acoustic recordings in the event of rain bouts, but we continued recording once the rain ceased. Acquisition of sound data was made with an Avisoft Ultrasound Gate 416 and Avisoft RECORDER running in a Toughbook laptop (Panasonic). Acoustic recording was conducted with a 16-bit resolution at a sampling rate of 250 kHz

Video analysis

Behaviors observed on video near the furled leaf were classified using a customized ethogram (Table 2.1; see Supplemental Material for video files of behaviors observed near furled leaves) that logged the type of approach behavior, the number of bats approaching the leaf, and the time of occurrence of each observation (i.e. time of behavioral event). We defined three categories for the type of leaf being monitored: i) previous roost - leaf in which bats were found roosting during the day; ii) night roost - available leaf where bats entered during the night at least once; iii) unoccupied leaf – suitable leaf not used as a night roost by bats. As part of our post-hoc analyses, we divided suitable leaves in two separate categories that reflect whether bats

entered a leaf but did not remain there through the morning (night roost) or whether the leaf remained unoccupied throughout the night (unoccupied leaf) despite exhibiting preferred structural characteristics.

Table 2.1. Ethogram for the behaviors scored when bats approached furled leaves.

Behavior	Description
Pass by	Flies across the recording view but does not approach furled leaf
Approach	Flies towards leaf. The offset of an approach was recorded when a bat was observed to fly towards the leaf and was positioned within at least 1m from the leaf
Hover	Flies towards the tip of the furled leaf and performs a suspended flight above the leaf directing the snout towards the roost entrance
Circling	Flies either from the base of the furled leaf or from above performing a circling flight usually in the form of a spiral directing the snout towards the leaf
Chase	Two or more individuals fly in close proximity, typically trailing one behind the other
Enter	Individual lands on the tip of the leaf and use wrist and ankle discs to move in direction to the base
Exit	Individual uses wrist and ankle discs to move towards the tip of the leaf, then pushes its body with the four discs towards the back and begins to fly.

We scored behaviors by observing videos at $\frac{1}{8}$ playback speed. When present, simultaneous video and audio recordings allowed us to link behavioral observations with the acoustic signals emitted by bats, and we noted the presence and type of social call associated with a behavioral observation. In our video recordings, bats within a group were not individually identifiable due to their rapid movements and small body size. Yet, our primary interest was in quantifying overall visitation to each leaf, regardless of the identity of the visiting bats; hence information about individual identity was not necessary. Behavioral data was obtained using the analysis software JWatcher v. 1.0 (Blumstein et al., 2007). Video scoring was carried out by the same person.

Acoustic repertoire

We visually examined social signals and categorized them according to the patterns observed in the call structure. We identified 6 call types (Fig. 1): (a) downward modulated frequency (INQ)- (previously described as inquiry call in Chaverri et al., 2010), (b) short quasi-constant frequency (SQCF) – short element of dominant frequency within 5kHz of start frequency (c) quasi-constant frequency (QCF) – dominant frequency within 5kHz of start frequency, (d) long upward modulated (LUM)- initial upward sweep followed by a quasi-constant frequency section, call usually occurred in bouts of between two to 5 calls (e) short upward (SUP)- concave downward sweep with positive slopes, and (f) short upward modulated (SUM)- concave upward sweep with positive slopes and step-like increases. Echolocation sequences correspond to low intensity broadband signals (Figure 2.1 g.). We characterized call structure by measuring signal parameters from power spectra, waveform and spectrograms. Visual examination of social calls and acoustic measurements were done using SasLab Pro v. 5.2.07 (Avisoft Bioacoustics). Call duration was measured from the waveform. Spectral properties were measured with a FlatTop window, 1024 point fast Fourier transform and 75% overlap, resulting in a frequency resolution of 244 Hz. Frequency parameters included: start (f_{start}), end (f_{end}), peak (f_{peak}), minimum (f_{min}) and maximum (f_{max}) frequency. Data of the frequency parameters was collected from the spectrogram using the automatic parameter measurements function. Peak frequency (defined as the frequency of highest intensity within the signal) was obtained from the power spectrum. We limited our measurements to the fundamental frequency in those social calls that exhibited multiple harmonics. To characterize call structure we also included calls that were recorded in the vicinity of a focal leaf but where no bats were observed in our video. Only calls with high signal-to-noise ratio were included in the analysis.

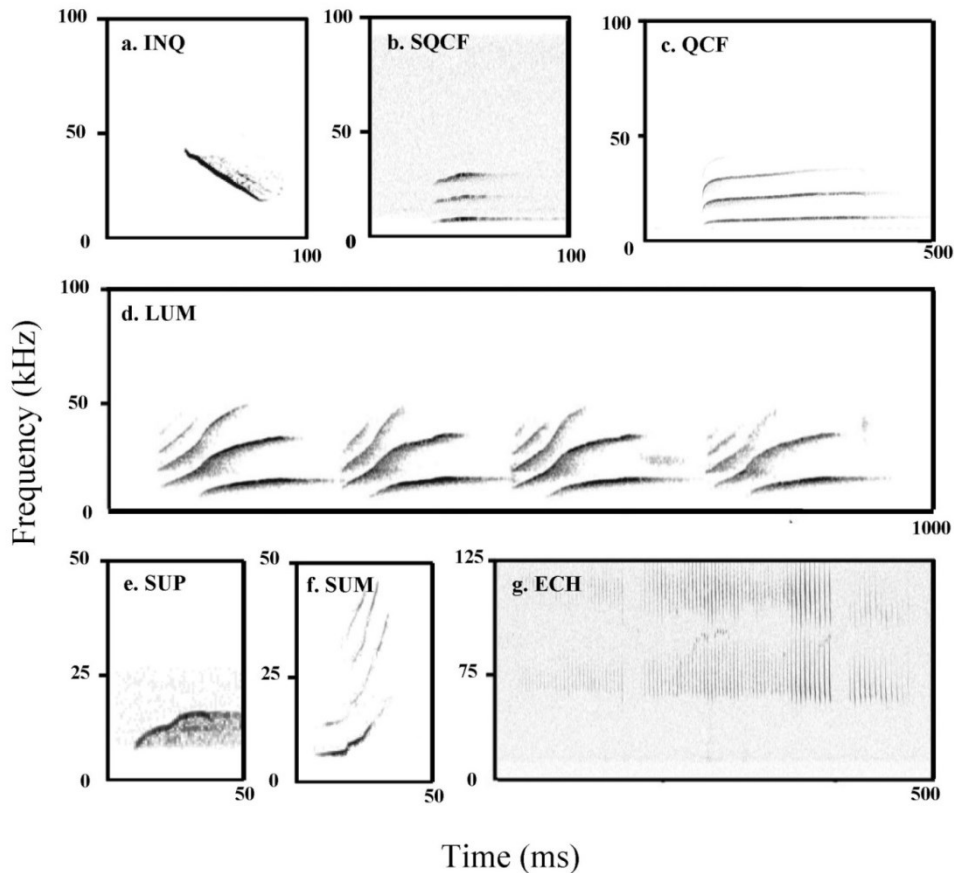


Figure 2.1. Spectrograms of the six social calls (a-f) and echolocation sequence (g) emitted by *T. tricolor* in the proximity of furled leaves. Frequency is plotted against time using a 1,024 FFT and a FlatTop window with 75% overlap.

Statistical analysis

To test whether the behavior observed in the vicinity of a leaf varied between leaf type, time or site, we fitted generalized estimation equations (GEE). Since we recorded multiple observations throughout the night for each leaf monitored, our data for each group lack independence. For analysis, we excluded those leaves where video recordings were interrupted due to extreme weather conditions or equipment failure, resulting in unequal sample sizes across groups. We accounted for differences in sampling effort, as well as lack of independence of multiple observations made at each leaf, in our GEE models. GEE allows us to account for the spatial and temporal correlation structure of the behavior recorded in the vicinity of furled leaves

by grouping observations into clusters that can be subsequently considered statistically independent. We assumed an autoregressive correlation structure and allowed repetition of focal leaf. In addition, we included group size as an offset term and acoustic recording duration (log sampling duration) to account for the variation of sampling effort between groups. The following variables were used as response terms: 1) number of visits per night, 2) duration of the approach behavior, which was defined as circling, hover or overall approach (sum of circling and hover), 3) the presence of social calls when approaching a leaf and 4) the rate of social calls recorded in the vicinity of a focal leaf. We estimated call rate by quantifying the total number of social calls recorded in the vicinity of monitored leaves, including those where bats were not captured in our video recordings. A Poisson error structure was used for the response terms, except for presence of social calls for which we used a Binomial error structure. Leaf type (previous roost, unoccupied leaf or night roost), site (SIR, TOR or TAP) and time (observations grouped in 1 hour time categories from 17:30-5:30 h) were used as explanatory variables. Models were fitted using the `geeglm` function of the `geepack` package (Højsgaard et al., 2006) in R (R Core Team 2014).

For those visits in which we recorded the presence of a social call, we examined whether the frequency of a particular call type was associated to 1) the number of bats visiting the leaf, 2) leaf type or 3) time of visit. The effect of each of these factors (call type, number of bats, leaf type and time) on the variability in number of calls recorded was examined using generalized linear models with a Poisson distribution.

Ethical note

The marking techniques used in this study allowed us to identify focal groups within a roost without disturbing individuals. Furthermore, during the length of our study we did not

identify any individual that showed signs of infection after implanting the PIT tags or attaching forearm bands, and all bats were observed to conduct normal flight behavior after being released. This study was approved by the Costa Rican authorities (MINAE; SINAC; reference no. ATM-ACOSA-001-01, 034-2012) and by the NDSU Animal Care and Use Committee (Protocol no. A11022, A12052).

Results

Approach behavior

We characterized the approach behavior of 14 groups of bats and a total of 38 leaves consisting of 456h of video and 297h of acoustic recording (see Appendix A for details on sample size). A total of 398 visits were recorded across all sampled leaf types and sites. We determined that the majority of visits were made by single individuals (76%); visits made by pairs of individuals were less common (17%) while visits of more than 2 bats flying together were rarely observed. Out of the 96 visits made by multiple individuals, 88% of these visits were characterized by the presence of a chase where typically one bat leads the flight throughout (Table 2.1). The majority of these chase events (70%) occurred when bats approached a night roost. Although we were not able to obtain reliable information about individual identity, the presence of a forearm band was visible in 13% of the visits. From this limited sample, we were able to confirm that both females and males approached leaves.

Out of the 38 suitable leaves monitored, 21 leaves were never occupied by bats during the night, 7 represented previous roosts and 10 were used as night roosts, 2 of which were also selected as day roosts. When comparing the number of visits made to each leaf type, bats were observed to visit night roosts more frequently than previous roosts or unoccupied leaves (GEE, Wald= 7.34, $p=0.007$; Figure 2.2). Further, variation in visitation frequency was more

pronounced among unoccupied leaves selected as night roosts (Figure 2.2 a). Bats at Sirena and Tapiria were observed to visit every recorded furled leaf within their roosting range at least once, with the most frequent visits to night roosts (up to 77 visits per night). The total number of visits was significantly different between sites (GEE, Wald= 23.39, $p < 0.001$). Tortuguero exhibited the lowest visitation frequency; half of the leaves monitored, including previous roosts and suitable leaves, were never visited. Further, the number of visits was independent of time (GEE, Wald=0.84, $p = 0.359$). Visiting behavior suggests that leaves are visited on multiple occasions during the night (Figure 2.2 b).

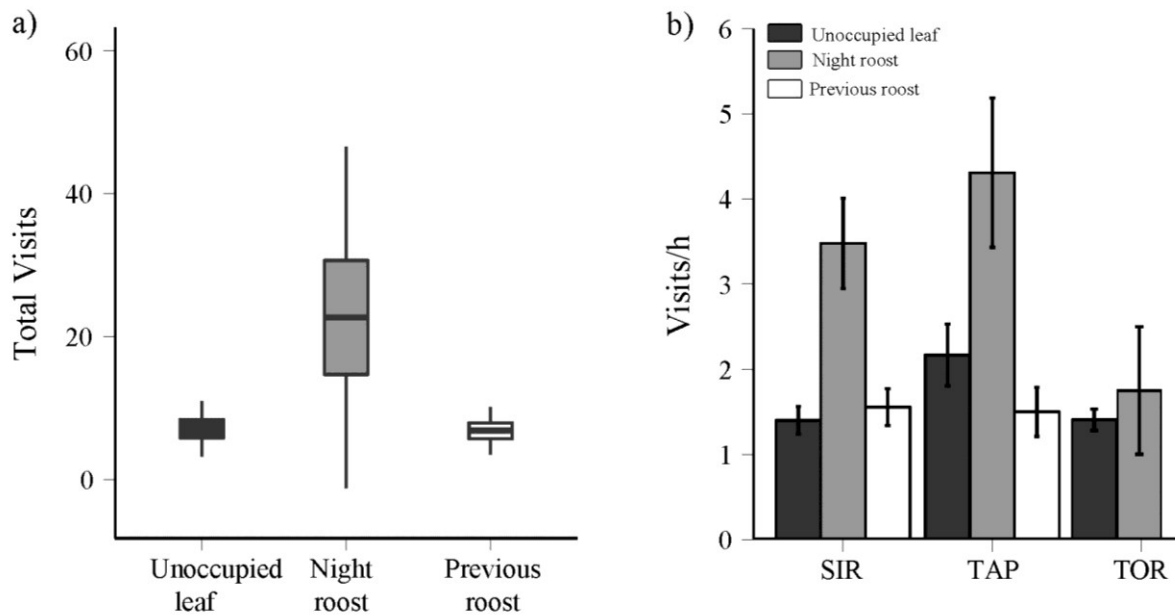


Figure 2.2. Monitoring frequency of focal leaves. (a) Variation in 1 visitation frequency between leaf type categories. Values for total visitation frequency were averaged (mean \pm SE) over leaf type category and pooled across sites ($n=38$, 21 unoccupied leaves, 10 night roosts and 7 previous roosts). (b) Number of individual bat visits per hour at three study sites. The number of visits per hour were averaged (mean \pm SE) over leaf category for each site ($n=29$, SIR= 13, TAP=9, TOR=7)

When visiting a furled leaf, approach behavior typically consisted of a circling flight followed by a short hover in close proximity to the leaf opening (Supplemental Fig. S1). The time spent in an approach event did not differ significantly between sites (GEE, Wald=5.56, $p=0.062$), however bats at Tortuguero were observed to spend less time flying within the proximity of furled leaves, whereas bats at Tapiria were spent more time during an approach event (Fig. 3). In addition, we found a significant interaction between leaf type and time category (GEE, Wald=6.61, $p=0.01$). Approach at night roosts lasted longer early during the night, while approach duration at suitable leaves was similar throughout the night. The time bats spent hovering on the leaf entrance did not differ significantly between sites or leaf type (Figure 2.3 b).

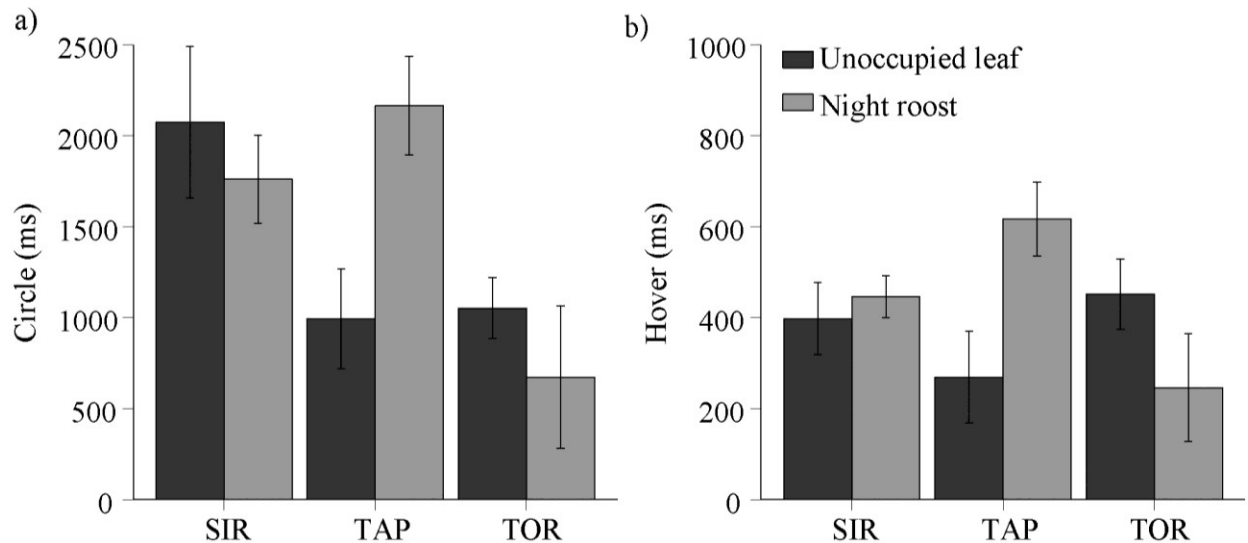


Figure 2.3. Duration of an approach event when visiting focal leaves. Approach is described as (a) circling (b) hover. Duration estimates of an approach event were averaged (mean \pm SE) over leaf type category ($n=23$, 13 unoccupied leaves, 10 night roosts) at three sites sampled.

Calling behavior

We analyzed a total of 722 social calls, which were visually classified into 6 call types. We excluded from the analysis the call type SQCF, as it was rarely recorded with sufficient signal-to-noise ratio. The vocalizations recorded were tonal signals with most energy from 10 to 32 kHz (Table 2.2).

Table 2.2. Mean \pm SE of call parameters of the social calls recorded at suitable roosting sites. Occurrence was estimated as a percentage from a total of 722 recordings with high signal-to-noise ratio.

	% occurrence	Dur	F _{start}	F _{end}	F _{peak}
INQ	42.4	39.0 \pm 0.6	31.7 \pm 0.3	15.9 \pm 0.2	32.1 \pm 0.2
LUM	33.2	175.5 \pm 1.7	15.9 \pm 0.2	22.8 \pm 0.3	16.3 \pm 0.2
QCF	9.6	374.1 \pm 13.9	10.5 \pm 0.2	12.9 \pm 0.3	10.7 \pm 0.2
SUP	6.8	47.1 \pm 3.0	17.1 \pm 0.4	20.3 \pm 0.3	17.7 \pm 0.6
SUM	6.5	27.5 \pm 1.2	11.2 \pm 0.2	20.4 \pm 0.4	11.6 \pm 0.2
SQCF	1.5	79.2 \pm 3.7	9.9 \pm 0.9	11.1 \pm 0.9	10.2 \pm 0.9

The most common vocalization types were INQ and LUM, which represented 42 and 33% of the total calls recorded, respectively. Overall, from the 156 events in which we recorded the presence of a social call, most signals were emitted by bats during a direct approach to the leaf (69%) or during a pass by behavior (28%). When examining whether the presence of a particular call type is associated with the leaf type visited, we found that LUM calls were most commonly recorded around unoccupied leaves (GLM, $p=0.045$), while no significant differences were detected for the other call types (Figure 2.4). The vast majority of social calls were recorded during visits in which an individual bat approached a leaf (90%), and no significant difference between call types was evident with respect to the number of bats that visited a leaf. We did not find any significant pattern in the presence of a particular call type with respect to time category; all call types were recorded throughout the night, although the number of calls increased between 0230 and 0430h.

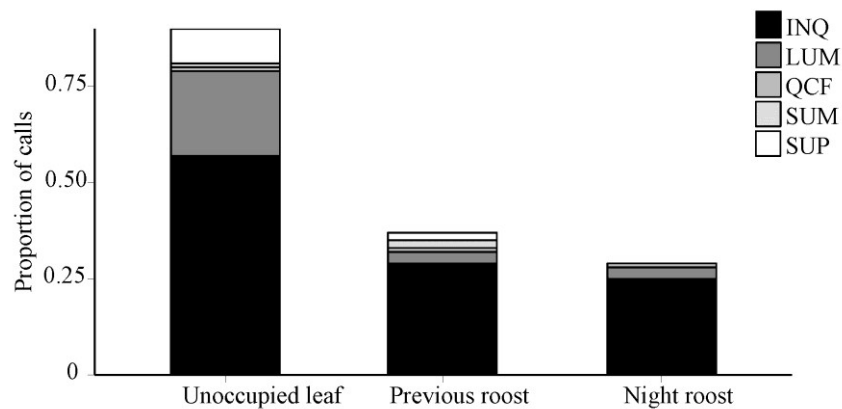


Figure 2.4. Proportion of social calls recorded at each leaf type. The different shades represent the frequency in which each call type was recorded. Call frequencies were pooled across sites.

The presence of social calls during a visit differed between leaf types. Based on our model, the probability of recording a call around unoccupied leaves was higher (GEE, Wald=3.89, $p=0.049$) compared to night roosts and previous roosts. We estimated that the presence of a social call made during a visit was of 76, 6, and less than 1% at unoccupied leaves, night roosts and previous roosts respectively. We did not find significance in the interaction term between time and leaf type was significant. At previous roosts and night roosts, the probability of recording a social call was similar throughout the night; however at unoccupied leaves this probability increased as the night passed (Figure 2.5).

Overall, call rate was similar between leaf types (GEE, Wald=0.86, $p=0.649$) and across sites (GEE, Wald=4.83, $p=0.09$). Call rate was significantly influenced by time (GEE, Wald=9.70, $p<0.001$). The number of calls recorded increased throughout the night, particularly at unoccupied leaves (Figure 2.6).

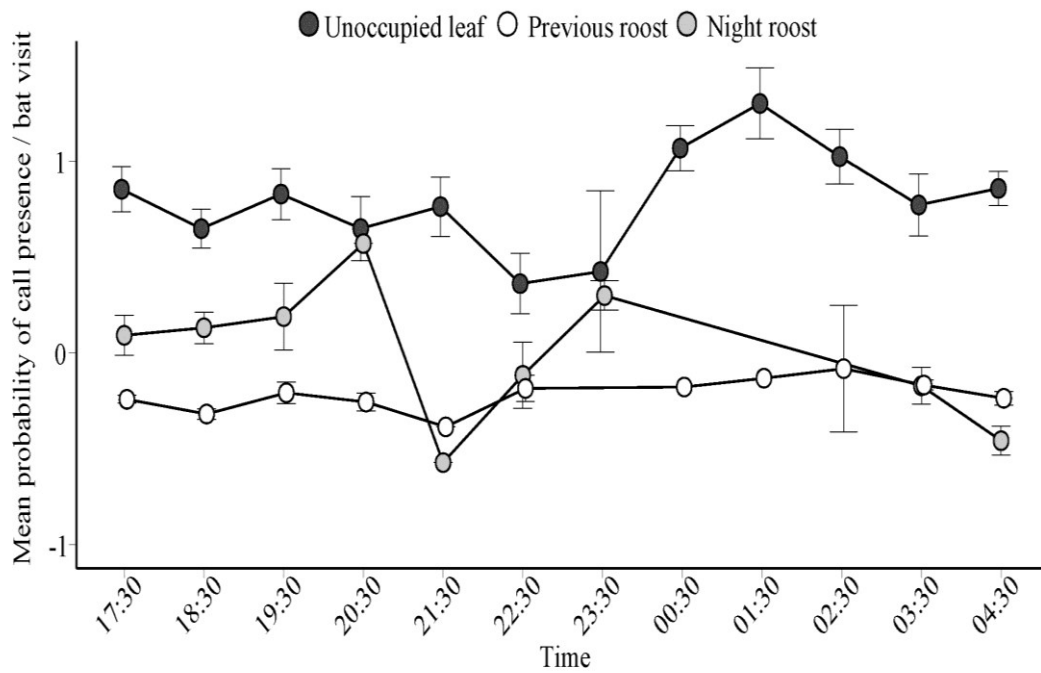


Figure 2.5. Estimated probability of recording the presence of a social call when bats visit a focal leaf as the night passed. Presence of social calls were estimated from bat visits in which continuous acoustic recording was available. Values were averaged (mean \pm SE) over leaf type category across sites (n=21, 12 unoccupied leaves, 5 night roosts and 4 roosts).

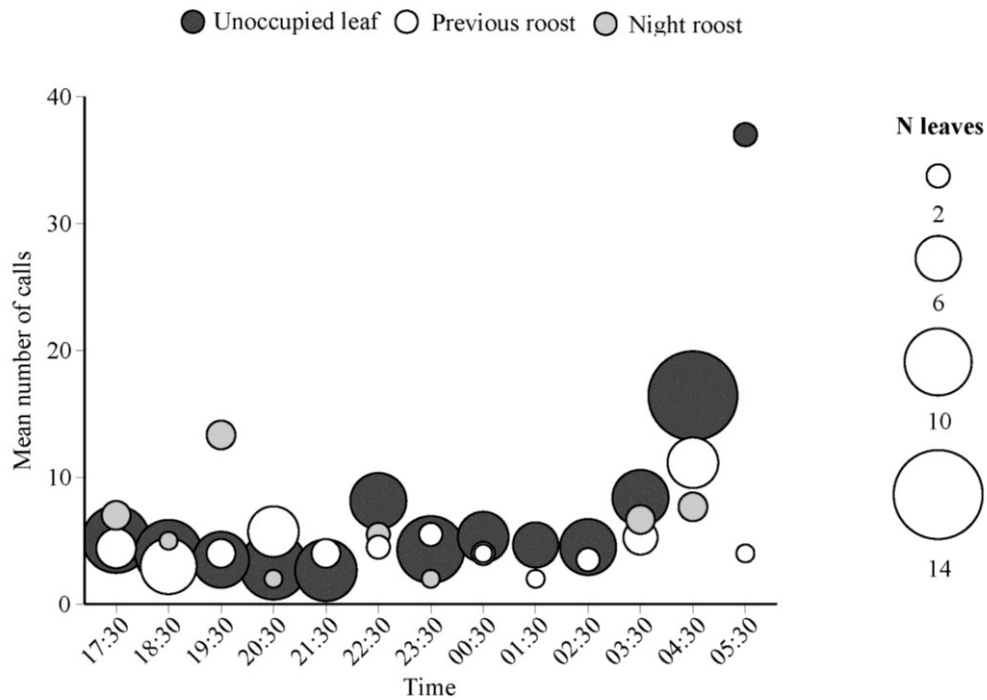


Figure 2.6. Frequency of social calls recorded at focal leaves in which continuous acoustic recording throughout the night was available. The number of calls recorded per hour was averaged over leaf type category (n=21, 12 suitable leaves, 5 night roosts and 4 previous roosts) and pooled across sites. Symbol size reflect the number of focal leaves.

Discussion

The purpose of our study was to characterize the patterns of roost assessment and the associated communication strategies of Spix's disc winged bat. Our approach of continuously monitoring roosting resources allowed us to: i) examine the behavior of *T. tricolor* in the vicinity of furled leaves, and ii) characterize the vocal repertoire of free-ranging bats during roost exploration. The need of *T. tricolor* to relocate new roosts on a daily basis led us to hypothesize that bats visit furled leaves within a groups' roosting range to assess its suitability as a day roost. In our study, we found that bats indeed approached furled leaves, although the frequency of these visits varied considerably across sites and types of leaves monitored. Given the social stability observed in *T. tricolor* we also hypothesized that bats would produce acoustic signals in the

vicinity of furled leaves as a behavioral strategy that would enable bats to regroup at some point during the night. Here we report the presence of distinct social calls and expand on our current knowledge of *T. tricolor*'s acoustic repertoire. Our approach in a natural setting is labor intensive, but provides the most realistic picture of natural patterns of behavior, and should be highly valuable for understanding the role of shelter stability in the evolution of the *T. tricolor* social system.

Monitoring of furled leaves

Our results show that one behavioral mechanism permitting *T. tricolor* to exploit a highly ephemeral roosting resource is continual assessment of potentially suitable roosts during nightly foraging bouts. We found that a focal suitable leaf was visited an average of ~7 times during the night. Because the dimensions of furled leaves do not remain constant (BKM, unpublished data), multiple visits within and between nights should allow individuals to evaluate the quality of a leaf as it develops. Alternatively, multiple visits may represent independent assessment from different individuals within a group. Although we were not able to reliably identify individuals, we did observe females and males approaching a focal leaf. This offers some evidence that multiple individuals visit furled leaves independently.

Approach behavior consisted of a close examination of both the furled leaf and its surroundings. The duration of the overall approach was dominated by a repeated circling flight around the leaf; on 87% of approach events, we also observed bats conducting a relatively short hover above the leaf opening. Both the inspection of leaf dimensions and surrounding vegetation presumably aids in the process of selecting the most appropriate roost on a given night. Bats would most likely choose leaves that will remain furled during the day and preferably those that offer less risk of predation. During the length of our study, we only observed two occupied

leaves that were partially unfurled by the end of the day, suggesting that in most cases, bats choose a leaf that remains furled for at least 24 hours.

When comparing the monitoring behavior of bats at different sites, we found that visitation frequency and approach duration was lower at Tortuguero compared to Sirena and Tapiria. Although our criteria for selecting focal leaves remained constant during the study, we encountered differences in plant density and composition across sites. While we mainly monitored *Heliconia* plants at Sirena and Tapiria, all focal leaves at Tortuguero were *Calathea*; patches of *Calathea* stands dominated Tortuguero's study site and *Heliconia* plants were rare. When estimating the rate at which leaves unfurl, Vonhof and Fenton (2004) found that *Calathea* were available as roosts for shorter periods of time than *Heliconia* spp. At Tortuguero, the high abundance of rapidly unfurling roosts may lead to a different optimal monitoring strategy compared to the other sites, where higher quality leaves are available at lower densities. Specifically, if a greater number of low-quality leaves must be visited to find a suitable roost at Tortuguero, a natural side effect would be a decreased number of visits to, and amount of time spent at, each leaf.

In our study we monitored furled leaves that we predicted to be suitable roosts. Half of these leaves were selected as night roosts by individuals of the focal group, two of which were also selected as day roosts. The use of night roosts is fairly common among bats (Kunz & Lumen, 2003), as they tend to offer strategic locations where animals are close to feeding locations, while also providing resting sites between foraging bouts (Knight & Jones, 2009). In general, night roosts were mostly used by one bat at a time, but ~ 37% of night roosts were occupied by 2 or 3 individuals simultaneously. Night roosts are potentially important in allowing individuals to regroup and engage in social interactions during the night.

While we originally anticipated that any selected roost would be occupied until the next evening (i.e., day roost), this was not the case. Alternatively, only two of our monitored leaves were used as day roosts, while a much larger number were used as night roosts. Although this post-hoc discovery was not ideal for characterizing day roost selection, this finding provides new insight into the nightly behavioral patterns of this species, as such common night roosting had not been previously reported. This finding also indicates that factors other than basic roost dimensions may be important for predicting day roost use.

Groups of *T. tricolor* typically exhibit small roosting home ranges (~0.4 ha), and previous work suggests that bats remain faithful to a habitat patch (Vonhof et al. 2004; Chaverri & Kunz 2011). Our results suggest that bats visit leaves within their roosting range regularly, including previous roosts that may no longer be suitable. This assessment of roosting resources offers some evidence that maintaining access to a minimum number of plants that will supply enough suitable roosting sites for both night and day shelters is a relevant aspect of *T. tricolor*'s roosting ecology as have been suggested in previous studies (Vonhof & Fenton, 2004; Chaverri & Kunz, 2011).

Communication strategies

The social stability of *T. tricolor* suggests the presence of a mechanism that allows individuals to encounter each other at some point during the night. In our study, we recorded a variety of distinct social calls emitted by bats that approached a furled leaf (Figure 2.1). These calls were emitted by bats during flight, which suggest that they are important in establishing or maintaining contact with conspecifics during nightly foraging and roost reconnaissance trips. While the fast flight behavior of *T. tricolor* means that tracking individuals and their associated patterns of call emission is particularly difficult, we can gain insight into the behavioral function

of these calls by examining their temporal and spectral structure (Kime et al., 2000; Morrill et al., 2013).

One critical factor in understanding the communication signals of any species is the environment in which the animal primarily lives (Ryan & Brenowitz, 1985; Bradbury & Vehrencamp, 2000). Groups of *T. tricolor* are usually found in clearings of secondary forests, where the surrounding vegetation is dense. Such an environment is challenging in terms of acoustic communication, as sound propagation will be hampered due to attenuation from scattering and deflection of acoustic signals (Wiley & Richards, 1978). From a signal design perspective, elements of *T. tricolor*'s vocal repertoire are well suited for long-range communication in such environments. Two calls in particular are likely candidates for locating group members during flight, namely QCF and LUM. These calls are relatively low in frequency and narrowband, which is ideal for longer transmission distance (Wiley and Richards, 1978; Morton, 1975). Also, the low frequency modulation of the LUM call coupled with its production in trill-like bouts (2- 5 calls) may enhance the receiver's ability to estimate the distance of the signaler (Naguib, 1995; Naguib, 2003). Overall, the LUM call type was the second most common signal recorded in our study, which likely reflects its relevance in facilitating group interactions during night activity.

Inquiry calls were the most common call emitted by bats during our study. Previous work has demonstrated that this call is part of a two-signal system that permits individuals to locate roosting group mates (Chaverri et al., 2010). Experiments have also revealed that inquiry calls encode individual signatures that can be discriminated amongst bats in flight (Gillam & Chaverri, 2012). Despite the common detection of inquiry calls, we did not record "response" calls, which are the second part of the signaling system that facilitates recruitment to roosts

(Chaverri et al., 2010). Because previous studies have only assessed diurnal calling behavior, use of the inquiry-response contact call system may be a strategy only used to relocate a roost within a very short period of time after a disturbance event. Yet, we do not exclude the possibility that this calling system is employed when selecting a day roost, since the number of day roosts we were able to record was small (2 day roosts).

The acoustic repertoire we describe in this study reveals that *T. tricolor* produces multiple structurally distinct signal types during flight (Fig. 1, Table 2). While elements of this calling system are particularly common to detect, some vocalizations were extremely rare. Given the observational nature of our study, we were not able to definitively link call types with specific behaviors, which would give insight into call function. All call types were recorded when bats flew in close proximity to a focal leaf; in general social calls were less commonly recorded in association with other behaviors, such as entering or exiting a roost. Finally, there was no association between the number of bats present and the types of calls produced (i.e. no calls were exclusively produced by solitary individuals or bats within a group). Further investigations, potentially employing playback experiments of social calls, might be valuable for elucidating the function of rare call types.

Despite the relatively small areas of habitat that we acoustically sampled, we report that bats regularly emit social calls during the night (Figures 4 and 5), indicating that actual call production rates across a group's entire home range are even higher. One possibility is that extensive social calling may facilitate group cohesion by advertising the location of suitable roosting sites within the group's home range. The use of contact calls to advertise roost location is common among bat species (e.g. tree-dwelling bats (Schöner et al., 2010; Furmankiewicz et al., 2011), crevice roosting bats (Arnold & Wilkinson, 2011), foliage roosting bats (Gillam et al.,

2013). Previous work on noctule bats has shown that locating a suitable shelter is not a trivial task, and that social cues aid in the process of roost selection (Ruczyński et al., 2007). Similarly, the monitoring behavior observed in *T. tricolor* indicates that locating and selecting a suitable roost may be energetically costly and that social behavior could greatly influence the ability of individuals to acquire roosting resources.

Social calls could also serve a role in delimiting the territory of a group. Because bats are probably dependent on a minimum amount of plants that will guarantee a reliable supply of roosts (Vonhof et al., 2004; Chaverri & Kunz, 2011) a benefit of grouping may be expressed by the ability of a social unit to monopolize a high quality habitat (Wilkinson & Boughman, 1998). Playback experiments would potentially help elucidate if acoustic signaling plays a role in group spatial delimitation.

Both our observations near potential roosts and the acoustic behavior of *T. tricolor* suggest that a behavioral mechanism that facilitates information transfer about suitable roosts is likely to be present in this species. We observed multiple bats approaching a leaf, seemingly as a chase. This behavior occurred throughout the night, providing evidence that bats encounter each other and possibly fly in close proximity repeatedly. Such encounters at suitable leaves may serve to assess roost quality, a process that may be mediated by group decision making. In addition, our results from those leaves in which continuous acoustic recording was available indicate that the overall rate of social call production increased with time, with more calls recorded closer to dawn. This pattern may be associated to the timing in which individuals regroup and select a day roost. Future research is necessary to address how bats decide which furled leaf to select as a day roost and whether this process is done as a group unit.

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III. GEOGRAPHIC VARIATION IN CONTACT CALLS EMITTED BY A LEAF-ROOSTING BAT SUGGEST DISTINCT MODES OF VOCAL TRANSMISSION

Introduction

Geographic variation in acoustic signals is known to occur in a wide variety of species (e.g. Mann & Lobel 1998; Irwin 2000; Höbel & Gerhardt 2003; Campbell et al. 2010) and can have important consequences for behaviors, such as mating (O'Loghlen, & Beecher 1997; Searcy et al. 2002; Danner et al. 2011), inter- and intra-specific interactions (Pasch et al. 2013). While most work addressing geographic variation in vocal signals has focused on bird song, which is used for mate attraction and territorial defense (Nowicki & Searcy 2002), substantially less attention has been given to calls with other types of social functions. In group living animals, vocal signals, known as contact calls, are a critical mechanism for mediating social interactions between group members (Cheney et al. 1996). Contact calls can facilitate social cohesion by promoting key group behaviors, such as coordination (Boinski and Campbell 1995; Townsend et al. 2011), conspecific spacing (Leighty et al. 2008) and advertisement of food and shelter locations (Valone 1996; Wilkinson & Boughman 1998; Chaverri et al. 2010; Arnold & Wilkinson 2011). Given the functional relevance of contact calls in grouping behavior, addressing the relationship between population dynamics and the patterns of contact call variation can provide important insight into the role of these vocalizations in social behavior.

Because contact calls typically enable individuals to obtain and share information of common interest to group members (Bradbury & Vehrencamp 2000), the frequency and strength of interactions within a group can influence how call structure varies within and between populations (McComb et al. 2000). Resident killer whales, for instance, form cohesive social groups and use contact calls with low intra-group call variation encoding information about

group membership (Ford 1991). Such “group calls” appear to be learned, as similarity in call structure between individuals within a pod is strongly predicted by the strength of social affiliation rather than relatedness alone (Deecke et al. 2010). Alternatively, in some species contact calls can show relatively high variability within a group but are consistent within individuals (e.g. primates, Gouzoules & Gouzoules 1990; pallid bats, Arnold & Wilkinson 2011). In this case, variation in acoustic features could reflect genetic relationships if calls are inherited traits (Forstmeier et al. 2009; Dreiss et al 2014). Geographic variation in contact calls could therefore be influenced both by degree of association between group members as well as by the mechanisms in which calls are transmitted in the population.

If vocalizations are culturally transmitted, meaning that individuals learn the acoustic features of a call, geographic variation is considered to be mainly attributed to both the process of signal copying and the scale of dispersal (Podos & Warren 2007). On the other hand, if differences in call structure are related to genetic factors, geographic variation may reflect patterns of gene flow across the landscape. Identifying the relative contributions of social and genetic factors influencing acoustic differentiation is difficult given that similar patterns can arise from both processes; acoustic differentiation in relation to geographic distance would be an expected outcome whether the features of a call are learned or genetically determined. However, historically isolated populations offer the opportunity to examine if long term genetic drift or selection influence divergence in a phenotypic trait, such as acoustic call structure (Nosil et al. 2009). Here, we examine the relationship between geographic variation of contact calls and genetic population structure of the disc-winged bat, *Thyroptera tricolor* separated by a geographical barrier.

Spix's disc-winged bats occur in neotropical humid lowland forests, exhibiting a wide distribution ranging from southern Mexico to southern Brazil (Wilson 2008). *T. tricolor* is characterized by the presence of discs on the thumbs and feet that enable bats to roost inside furled musoid leaves; this feature also forces individuals to relocate new roosts every day due to the ephemerality of these leaf shelters (Findley & Wilson 1974). Despite the regularity in which bats switch roosts, *T. tricolor* form stable group associations lasting up to 22 months (Chaverri 2010). This roosting ecology is thought to be linked to a variety of features of the social behavior of this species, including their communication strategies (Chaverri et al. 2010; BKM and EHG, unpublished data). Earlier work on the signaling behavior of these bats identified the use of a call and response system enabling individuals to actively recruit group members to known roosts (Chaverri, et al. 2010). In addition to these two signals, a number of contact calls used by disc-winged bats during roost assessment were recently documented, suggesting that to maintain contact with conspecifics, *T. tricolor* uses a combination of acoustic signals during night activity (BKM and EHG, unpublished data). Among the acoustic signals produced by these bats, two calls are particularly common near furled leaves: a short broadband call previously labeled as an inquiry call (INQ), and a long narrowband signal typically produced in bouts of two to five calls (LUM). Both call types are known to exhibit low stereotypy; INQ calls are highly variable within groups encoding information about individual identity (Gillam & Chaverri 2012) and while no study has addressed acoustic variation of LUM signals, differences in LUM call structure are readily apparent (BKM and EHG, unpublished data).

To study if patterns of acoustic variation are congruent to genetic distance, we compared allopatric populations separated by a geographical barrier, the volcanic mountain range of Costa Rica. We sampled multiple social groups in protected areas located along the Pacific and

Caribbean slopes, and estimated population genetic structure using nuclear microsatellite loci. We predicted that limited gene flow between populations (due to the presence of the volcanic range) would result in population sub-structuring, and that geographic variation in INQ and LUM calls would match this pattern of genetic differentiation. *T. tricolor* shows all-offspring natal philopatry (Chaverri and Kunz 2011), and social groups consists primarily of females and her offspring (Buchalski et al. 2014) that are loyal to a patch of roosting resources (Vonhof & Fenton 1999; Chaverri and Kunz 2011). Given these patterns of natal philopatry and spatial stability, we expect a gradual divergence in contact call structure within regions.

Methods

Study sites and data collection

Field work was conducted between 2011 and 2012 at four sites within three protected areas in Costa Rica: Corcovado National Park located in the south Pacific coast, Tortuguero National Park and Tapiria National Reserve located in the northeast Atlantic region. Sampling sites were Sirena (SIR, 8.48° N, -83.59° W), San Pedrillo (SAP, 8.62° N, -83.73° W), Cuatro Esquinas (TOR, 10.54° N, -83.50° W) and Tapiria (TAP, 10.53° N, -84.03° W) field stations (Figure 3.1). The sites in the Pacific, SIR and SAP are connected by continuous forest, belonging to Corcovado National Park, while TAP and TOR sites are separated by agricultural and human development. At each of these sites, we captured bats roosting in furled *Heliconia* spp. and *Calathea* spp. leaves by pinching the top of the leaf and directing individuals into a cloth holding bag. Upon capture we recorded standard measurements (body mass, forearm length, sex, reproductive state and age) and obtained tissues from the wing membrane with a 4mm medical biopsy punch.

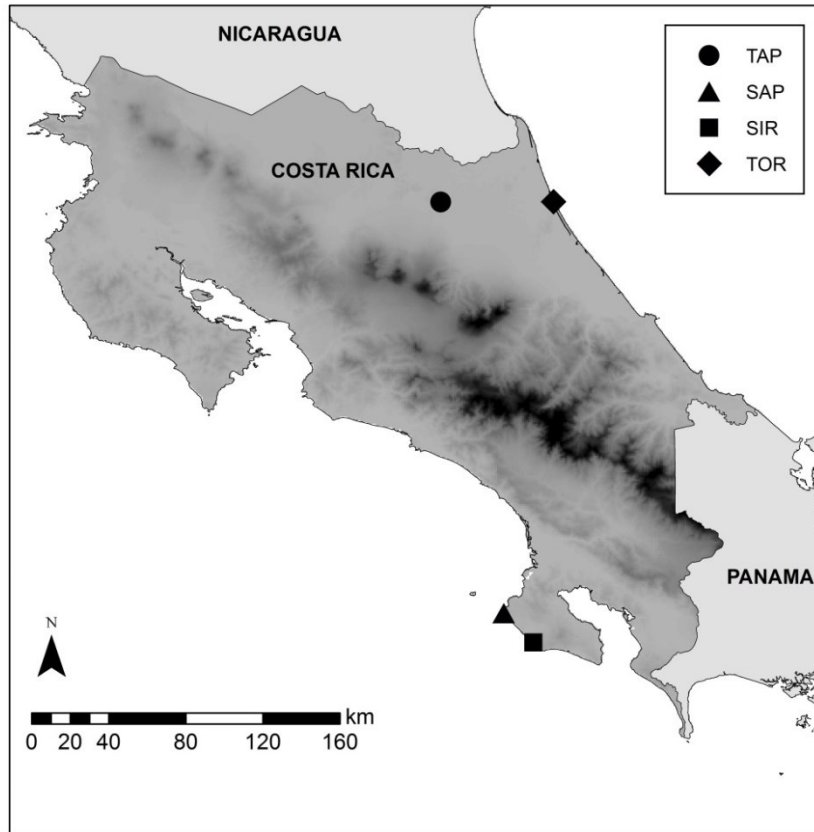


Figure 3.1. Geographic location of 4 sampling sites (solid symbols) representing the Caribbean and Pacific slopes in Costa Rica. Dark shading illustrates the elevational gradient determined by the volcanic mountain range.

Tissue samples were stored in lysis buffer until DNA extraction. Vocalizations of free-ranging bats were recorded during the night by placing an ultrasonic recording system and a video camera in the vicinity of a previously-used roost leaf, as well as available leaves that might serve as potential day roosts. Recordings were made using an Avisoft condenser microphone CM16 aimed at the distal end of the furled leaf. Vocalizations were sampled at a rate of 250 kHz and a 16-bit resolution with an Avisoft Ultrasound Gate 416 and downloaded to a Toughbook laptop (Panasonic) using Avisoft RECORDER. Acoustic monitoring lasted between 4-11 hours each night. At each site, we monitored three to five independent groups for at least 3 nights, resulting in a total of 17 groups sampled. Our approach to sampling acoustic signals was

restricted to free-ranging animals since bats do not produce LUM calls in enclosed environments. Given the fast movements and small body size of bats, we were not able to assess individual identity, therefore our analysis is restricted to a comparison of contact calls produced by different groups. Sample size (N groups) for acoustic and genetic data for each site is summarized in Table 3.1.

Table 3.1. Number of groups, intragroup distance (m), and acoustic (mean \pm SE) and genetic sample sizes for each site sampled at the Caribbean and Pacific slopes in Costa Rica.

Region	Site	N _{Groups}	Intragroup distance		N calls		N _{tissue samples}
			min	max	INQ	LUM	
Caribbean	TAP	3	201.2	808.3	19.3 \pm 10.8	15 \pm 8.7	12
	TOR	5	114.4	498.6	19 \pm 3.4	17.2 \pm 6.8	28
Pacific	SIR	5	102.6	1129	14.4 \pm 3.7	23.8 \pm 12.8	24
	SAP	4	216.5	1467.4	26.5 \pm 14.5	8.7 \pm 0.9	23

Analysis of social calls

The spectral properties of INQ and LUM calls were measured using SasLab Pro v.5.2.07 (Avisoft Bioacoustics). Call duration (dur_c) was measured from the waveform. Peak frequency (f_{peak}), defined as the frequency of highest amplitude within the signal, was obtained from the power spectra. Frequency parameters at the start (f_{start}), end (f_{end}), minimum (f_{min}) and maximum (f_{max}) frequency of the signal were measured from the spectrograms using the automatic parameter routine in SasLab Pro (Fig. 2). In addition, we described signal contours by partitioning call duration into 50 sections, resulting in 49 measures of peak frequency along the fundamental frequency of the signal. Measurements were done using a FlatTop window, 1024 point fast Fourier transform and 75% overlap. This resulted in a frequency resolution of 244 kHz and a time resolution of 1.03 ms. We calculated slope and concavity at each section as $(freq_{t1} - freq_{t0}) / (t1 - t0)$ and $(slope_{t1} - slope_{t0}) / (t1 - t0)$ respectively, where t_0 corresponds to the start time of a

given section and t_l the start time of the following section (Gillam & Chaverri 2012). For analysis, we report minimum, maximum, median and 95 percentile of the slope (S_{\min} , S_{\max} and S_{95} percentile) and concavity (C_{\min} , C_{\max} and C_{95} percentile). Measurements were only taken from the fundamental frequency, in which most of the energy is concentrated. For analysis, we only included those calls that exhibited high signal-to noise ratio.

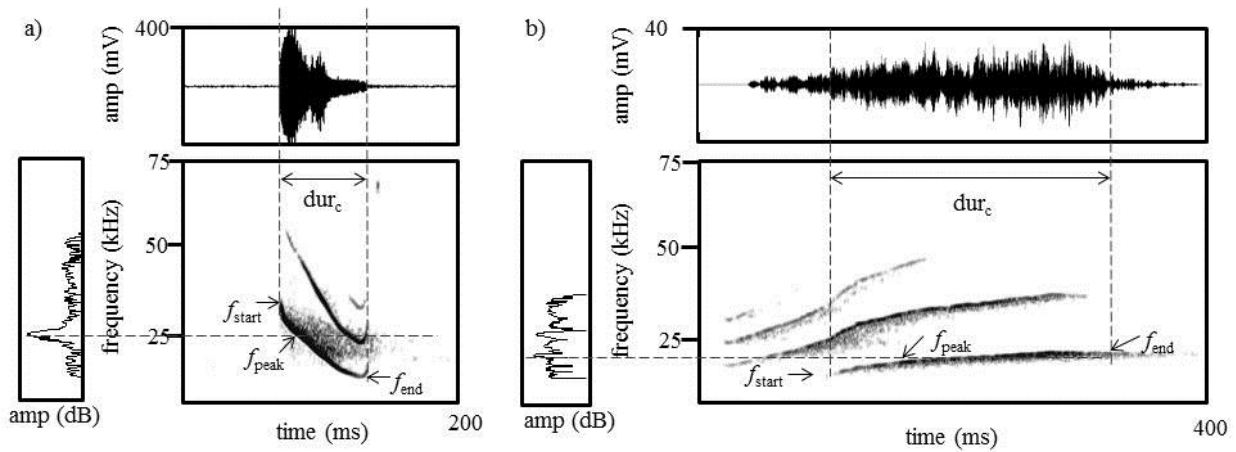


Figure 3.2. Spectrograms of contact signals a) INQ and b) LUM produced by *T. tricolor* during flight. Oscillogram (top panel) and power spectrum (left panel) are also depicted. Acoustic measurements were taken from the fundamental frequency.

To describe call variation of INQ and LUM signals we first reduced the number of acoustic parameters to a subset of uncorrelated variables derived from a principal component analysis (PCA). Principal components were extracted using a varimax rotation method, and for subsequent analysis we excluded those components that contributed less than 10% of the total variance. To examine effects of region and site on variance of signal structure, we used a permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations based on pairwise Mahalanobis distance derived from the retained PC scores (Adonis function, VEGAN package in R, Oksanen et al. 2007). To further examine the patterns of acoustic variation in signal structure within regions, we conducted Mantel tests to address the relationship

of geographic distance and acoustic distance of INQ and LUM calls. Geographic distance between groups was estimated using straight-line intragroup distances, and Mahalanobis distance was used to describe acoustic distance between groups.

Genotyping

We extracted DNA from a total of 86 bats from 17 groups using a Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Chatsworth, California). We amplified 10 of the 11 microsatellite primers (primers for locus 6 failed to amplify readily scorable products) developed for *T. tricolor* (Vonhof *et al.* 2001). The M13 method (Schuelke 2000) was followed to fluorescently label loci. We used a step-down thermocycler profile (McCulloch and Stevens 2011) for the polymerase chain reaction (PCR) with the following conditions: 95 °C (3 min), 12 cycles at 95 °C (20 sec)/ initial annealing temperature (20 sec)/ 72 °C (30 sec), decreasing 2 °C every cycle, 24 cycles at 95 °C (20 sec)/ final annealing temperature (20 sec)/ 72 °C (30 sec), and a final extension at 72 °C (7 min). Initial and final annealing temperatures were optimized for each locus, with temperatures ranging from 62-54 and 54-44 °C respectively. The PCR was performed in a total volume of 12.5 µL with a final concentration 1 X PCR Buffer, using 1-4 ng of template DNA, 1.25 units of Taq DNA polymerase, 0.25 µM primer concentrations, 2 µM dNTPs and ultrapure water to volume. Products were sized on an ABI PRISM 3100-Avant (Applied Biosystems), and genotypes were scored with GeneMapper version 4.0 (Applied Biosystems).

Analysis of population differentiation

We estimated genetic diversity by quantifying the number of alleles, observed (H_O) and expected heterozygosity (H_E) within each region. Genotypic proportions were evaluated for significant deviations from Hardy-Weinberg and linkage equilibria using Genepop 4.2 (Raymond & Rousset 1995). We estimated the frequency of possible null alleles using CERVUS

3.0 (Marshall et al. 1998). The analysis was also conducted with sites as population samples to confirm that significant deviations were not a result of substructure within the study sites. A sequential Bonferroni correction for multiple tests (Rice 1989) was applied to assess significance.

To test for genetic differentiation among populations we estimated genetic distance based on F -statistics. Pairwise F_{ST} comparisons between groups were computed following Wier & Cockerham (1984) by randomization (10,000 permutations) using ARLEQUIN version 3.5 (Excoffier & Lischer, 2010). We conducted analysis of molecular variance (AMOVA, 100,000 permutations) using a hierarchical approach by including region and site as nested levels of differentiation (amova function, PEGAS package in R, Paradis 2010). In addition, we calculated a distance matrix on Nei's D standard distance (Nei, 1972) and generated a graphical representation of the genetic distance among groups using the UPGMA algorithm (agnes function, CLUSTER package in R, Maechler *et al.* 2015).

Population genetic structure was also inferred using a model-based clustering method implemented in the program STRUCTURE version 2.3 (Pritchard et al. 2000). To test for the number of populations (K) and the assignment probabilities of individuals to K populations, we ran the model assuming admixture and no prior knowledge of sampling origin. Parameters were set on a Markov chain Monte Carlo of 500,000 and a burn-in period of 100,000 for 10 replicate runs. The number of simulated K values ranged from 1 to 17 corresponding to the number of groups sampled.

Comparisons of acoustic, genetic and spatial structure

We examined the relationship between acoustic, genetic and spatial structure using redundancy analysis (RDA). RDA uses a multivariate linear regression technique to explore the

relative contribution of distinct sets of explanatory variables on genetic differentiation. With this approach, we are interested in addressing three questions. First, is intragroup acoustic variation in INQ and LUM calls correlated to the patterns of genetic distance? Second, does spatial structure, represented by the intragroup geographical distance between populations sampled across the two slopes of the mountain range, affect acoustic variation? Third, is there a correlation between acoustic variation of each contact call and genetic distance while taking into account the geographical variation due to spatial structuring of the sampled populations?

In our models, the dependent variables corresponded to acoustic variables describing INQ and LUM call structure. The acoustic variables used in this analysis represent a subset of uncorrelated variable combinations derived from a PCA. Here, all parameter measurements were included in the PCA, and using the same criteria for describing call variation (explained above) we retained four and three axes for INQ and LUM call types respectively. The explanatory variables corresponded to the genetic distances and spatial structure of the populations sampled.

Pairwise F_{ST} values were ordinated using a principal coordinate analysis (PCoA) and we used the broken stick criterion to determine the number of axes to retain for analysis (pcoa function, APE package, Paradis et al. 2004). To assess the effect of spatial variation, we constructed a distance matrix using straight-line intergroup geographical distances. For each group we first estimated roosting range based on minimum convex polygons created from the locations in which bats were found roosting across multiple nights and used the MCP centroids to build the pairwise distance matrix. Because our sampling scheme consisted of groups clustered within sites and across regions, geographical distance was modelled using Moran's eigenvector-based maps (MEM). This was done using the principal coordinates of neighbor matrices method (PCNM; Borcard and Legendre 2002) to better represent the spatial clustering

of groups. We computed the Euclidean geographical distance between groups and used the ‘pcnm’ function in R (PCNM package, Legendre *et al.* 2013) with an *a priori* threshold value for truncation that represents the minimum distance between sites in our study (20 km). Out of the 15 axes computed by the PCNM procedure, we retained 3 eigenvectors following the forward selection criteria (Blanchet *et al.* 2008). The selected eigenvectors represented the spatial axes used as explanatory variables. Using RDA (1000 permutations) we explored the amount of variance explained by: i) genetic distance, ii) spatial structure and iii) the conditional variance of genetic distance when controlling for spatial structure. Variance partitioning estimates were computed following Peres-Neto *et al.* (2006) using the rda and varpart functions (VEGAN package, Oksanen *et al.* 2015). Analyses were conducted for the entire population and separately for the Pacific and Caribbean population sets. All analyses were performed using the R framework v. 3.1.3 (R core Team 2015).

Results

Geographic variation in contact call structure

We recorded a total of 533 contact signals, 299 and 234 of which corresponded to INQ and LUM calls, respectively. We excluded from our analyses those groups in which we obtained less than 10 high quality calls per signal type. Thus, our final data for acoustic comparisons was comprised of 15 groups for each call type. Call variation was estimated from 4 and 3 principal components for the INQ the LUM data, respectively explaining 80.9% and 79.5% of the variation. Factor loadings for each PC axis are reported in Appendix B. Significant variation between regions was found in call structure of INQ ($R^2=0.17$, $F_{1,11}$, $p=0.035$) and LUM ($R^2=0.59$, $F_{1,11}$, $p<0.0001$) signals. However, there were no significant differences in either INQ or LUM calls within sites. The variation in call structure represented by the first and second

components of the PCA showed different patterns for INQ and LUM calls. For INQ calls, there is extensive overlap in the acoustic parameters denoted by PC1, which captures most of the parameters describing call contours (Figure 3.3a). Major differences in INQ call design among sites are represented by PC2; INQ calls from one site in the Caribbean, TOR, exhibit an increase in peak, start and max frequency compared to the other three sites. In contrast, distinct clustering of calls by region and high overlap between sites is evident for LUM calls (Figure 3.3b). Here, frequency parameters (peak, start and minimum) represented in PC1 describes the acoustic differences between the regions.

The relationship between acoustic variation and geographical distance within regions was further explored using Mantel tests (Figure 3.4). These analyses revealed a significant correlation between Mahalanobis distance of INQ calls and geographic distance in the Caribbean region (Mantel test, $R=0.57$, $P=0.033$). INQ calls were not influenced by geographic distance in the Pacific population. For LUM calls, the correlation between these variables was weak and not significant for either region.

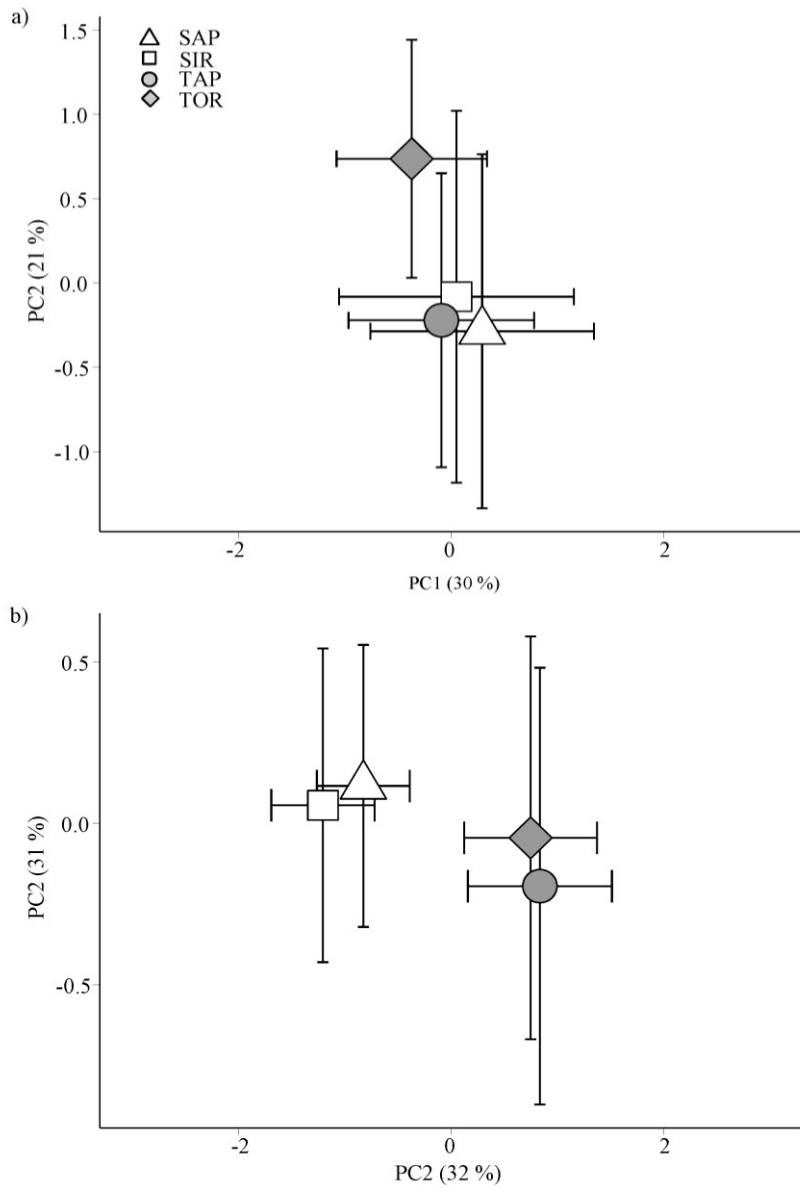


Figure 3.3. Plots of INQ (a) and LUM (b) calls representing mean scores derived from the first (PC1) and second (PC2) principal component axes for each site and the associated standard deviations. Shaded symbols correspond to the Caribbean sites and open symbols to the Pacific sites.

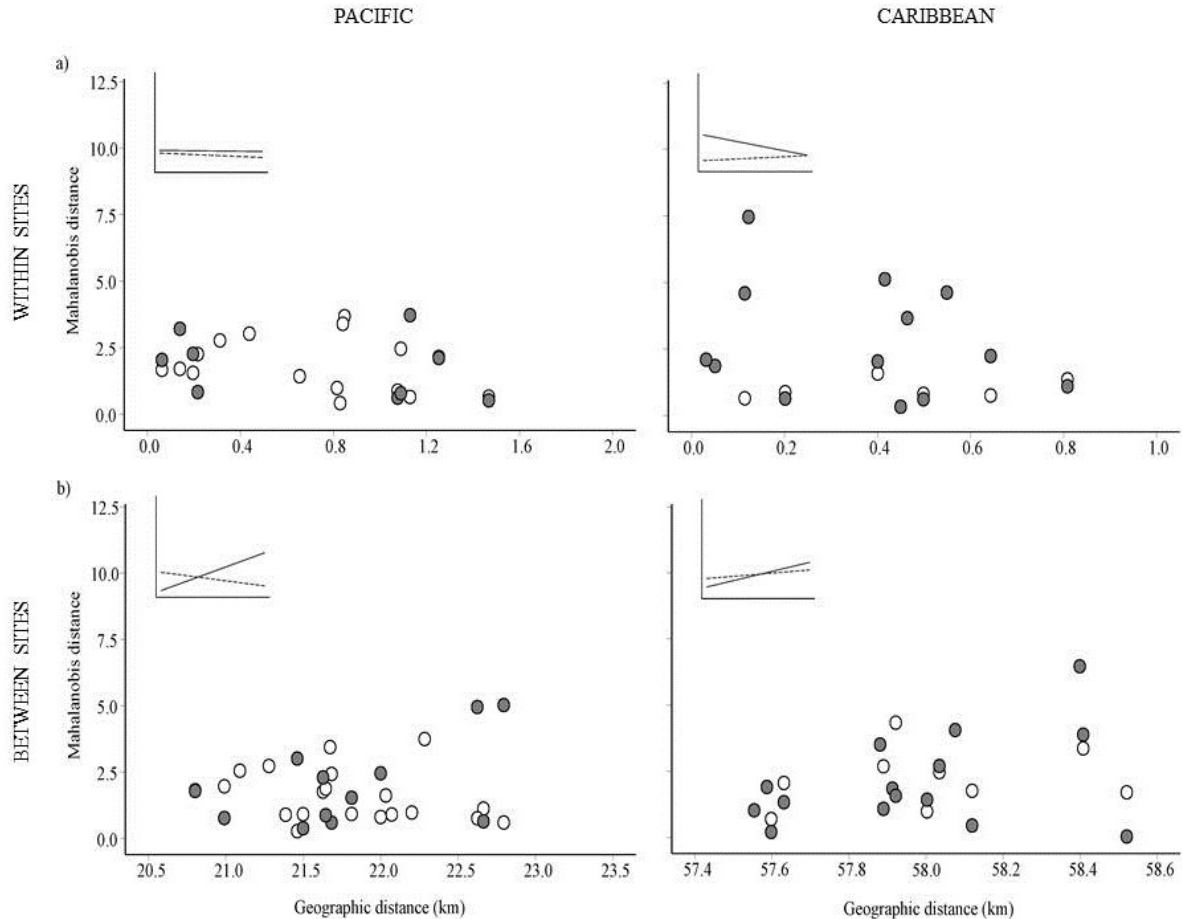


Figure 3.4. Relationship between acoustic distance (Mahalanobis) and geographic distance denoting group pairwise comparisons for a) within sites and b) between sites for the Pacific (left panels) and the Caribbean (right panels) populations. Open symbols correspond to pairwise comparisons for the INQ call set whereas shaded symbols correspond to the LUM call set. The inset plots indicate the slopes for the INQ (dotted lines) and LUM (solid lines) calls.

Microsatellite variability

The number of alleles per locus varied greatly, ranging from 4 (Tt34) to 20 (Tt10), and an average of 11.4 alleles per locus; observed per-locus heterozygosity ranged from 0.32(Tt4) to 0.93 (Tt8), with an average of 0.67. At regional and site levels, we detected deviations from Hardy-Weinberg for 4 loci (Appendix C). In addition, 3 loci (Tt10, Tt17 and Tt34) exhibited high null frequencies (>0.10). Therefore, these loci were excluded in the following analyses.

Population differentiation

Using a hierarchical AMOVA to partition the effects of region and sites on genetic differentiation, we found that both levels significantly influence genetic variation. We found that 24.1 % ($p < 0.001$) of the variance could be explained by region and 25.9 % ($p = 0.016$) by the four sites. We further investigated population differentiation by inferring genotype assignment with the Bayesian-based clustering program STRUCTURE. Population substructure was detected among our samples, indicating ($K=2$) as the most likely number of populations (Figure 3.5b). Further, in accordance with the STRUCTURE results, the UPGMA tree revealed the presence of two main clusters (Figure 3.5a). These two clusters are mainly represented by the two geographic regions; however groups within the TAP site were assigned to both populations.

Variation partitioning of genetic and spatial structure

We examined the relative contributions of genetic and spatial distance to the acoustic variation in contact calls of *T. tricolor* using a RDA variance partitioning approach. These analyses focused on determining how much of the variation in call structure is explained by the spatial structure of the populations sampled vs the effect of genetic distance. To distinguish between the patterns of variation given call type, the RDA was conducted separately for INQ and LUM matrices (Table 3.2). For the entire population, the variables describing genetic distance explained most of the variation in acoustic differentiation (~30%), resulting in a small proportion of the variation explained by the spatial predictors. While no statistical support was evident for either the genetic or spatial parameters explaining variation in INQ calls, these predictors significantly contribute to the variation of LUM calls for the entire population (Table 3.2). Variation in LUM calls is prominently explained by genetic distance when controlling for the effect of spatial variables (36%, $P=0.01$).

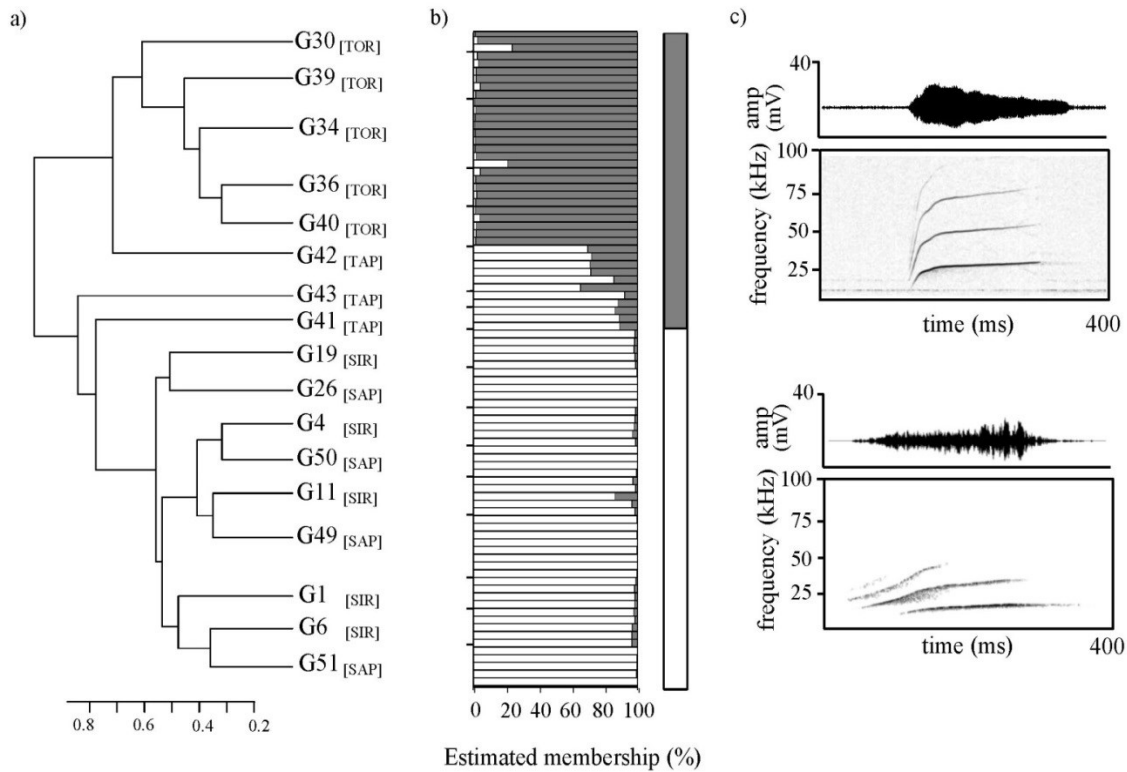


Figure 3.5. (a) Unrooted UPGMA tree based on Nei’s D_s pairwise comparisons over 7 loci. (b) Estimated individual membership to population gene pools by the program Structure. Individuals are represented by horizontal bars and the height of each color corresponds to the assignment probability to the two gene pools indicated by color shading. Individuals are arranged to correspond to the group and site labels in (a). Geographic origin in accordance with acoustic variants is illustrated by the shaded bar (grey: Caribbean, white: Pacific). (c) Spectrograms of the two signal variants of LUM calls corresponding to the geographic locations of populations (top: Caribbean, bottom: Pacific).

The RDA conducted separately on the Pacific and Caribbean populations highlight distinct patterns of variation for INQ and LUM contact calls. For the LUM call set, we found no statistical support for the contribution of the predictors on call structure on the Pacific or Caribbean populations, suggesting that within regions calls do not exhibit a clear pattern of differentiation that matches either geographic or genetic distance. Despite the lack of statistical significance, results for INQ calls indicate that genetic distance explains most of the variation in INQ call structure within the Pacific (44%, $P=0.087$) and the Caribbean (58%, $P=0.051$)

populations. While spatial variables do not contribute to the variation in INQ calls in the Pacific population, call structure within the Caribbean region was significantly influenced by the spatial predictors (44%, $P=0.015$).

Table 3.2. Results of the RDA variation partitioning analysis estimating the effect of spatial structure and acoustic differentiation of INQ and LUM calls on the genetic distance of *T. tricolor* populations. The adjusted ($R^2_{Adj.}$) variance and P -values were estimated for the overall population and separately for the Pacific and Caribbean populations. Estimates of variation are denoted by: [G]= proportion explained by genetic distance; [S]= proportion explained by spatial variables; [G|S]=unique fraction of variation explained by genetic distance when controlling for spatial variables; [S|G]= unique fraction of variation explained by spatial variables when controlling for genetic distance; [G+S]= shared variation explained by both genetic and spatial predictors.

Effects	INQ		LUM	
	$R^2_{Adj.}$	P	$R^2_{Adj.}$	P
Overall				
[G]	0.33	0.389	0.31	0.025
[S]	0	0.458	0.16	0.019
[G S]	-0.10	0.641	0.36	0.01
[S G]	-0.14	0.729	0.21	0.026
[G+S]	0.13		-0.06	
Pacific				
[G]	0.42	0.087	0.26	0.603
[S]	-0.06	0.628	-0.11	0.107
[G S]	0.37	0.226	0.39	0.130
[S G]	-0.11	0.616	0.03	0.132
[G+S]	0.05		-0.13	
Caribbean				
[G]	0.58	0.051	-0.18	0.877
[S]	0.44	0.015	-0.06	0.668
[G S]	0.07	0.654	-0.32	0.916
[S G]	-0.07	0.851	-0.21	0.845
[G+S]	0.52		0.14	

In summary, when considering the overall pattern of acoustic variation for the entire population, the RDA results show that both spatial variables and, to a greater extent, genetic distance, significantly explain the variation in acoustic differentiation only for the LUM call set. In contrast, when examining geographic variation of LUM calls within regions these predictors fail to significantly explain the patterns of acoustic differentiation of this signal. Acoustic divergence in INQ calls within the Caribbean region is however associated to genetic and geographic variables, providing evidence for discordance in the patterns of geographic variation of the two call types examined.

Discussion

The main purpose of this study was to determine whether geographic variation in contact calls produced by *T. tricolor* are congruent with genetic differentiation of two populations separated by a geographic barrier. We found support for the presence of acoustic divergence in concordance with genetic distance for one of the two contact calls examined. Our findings also reveal distinct patterns of acoustic and genetic differentiation within the two regions sampled, suggesting distinct mechanisms by which the two acoustic signals studied are transmitted across populations of Spix disc-winged bats. These findings are discussed with respect to the role of limited dispersal in population differentiation and the associated consequences in variation of social calls in *T. tricolor*.

Evidence for call divergence

We predicted that the patterns of acoustic differentiation in two contact calls regularly emitted by *T. tricolor* would be driven by the restricted connectivity between populations separated by a geographic barrier. Specifically, we expected to find greater acoustic differentiation between regions than within regions. Our results indicate that there is divergence

in call structure between the two regions sampled; however, we found a discordant pattern of geographic variation between the two types of contact calls studied. While the presence of LUM call variants is clearly delimited by the geographic barrier (Figure 3.3 b and Figure 3.5 c), evidence of divergence in the INQ call type is not; alternatively, one site (TOR) exhibits substantial differentiation from calls recorded in the other three populations (Figure 3.3 a). The distinct pattern of geographic variation between call types was also evident when comparing call structure within regions. We only found a correlation between acoustic distance and geographic distance in INQ calls from the Caribbean inter-group comparisons, which is the region that includes the highly divergent TOR site.

Call divergence between regions could be partially attributed to environmental factors shaping the features of the signals. Our study focuses on acoustic signals regularly emitted by bats during flight near suitable roosting site; these calls likely play a central role in facilitating social encounters among group members that are physically apart during foraging bouts (BKM & EHG, unpublished data). Similar to contact calls emitted by other species, the acoustic properties of these signals most likely reflect adaptation to the local environment to maximize transmission within the signaling habitat (Wiley & Richards 1978; Slabbekoorn & Smith 2002; Baker 2006). The two call variants of LUM vocalizations may be driven by environmental differences between regions affecting sound propagation. Further research addressing the influence of variables such as temperature and humidity on propagation of these signals would be necessary to determine whether the acoustic features of the call variants optimize signal transmission in the habitat occupied by *T. tricolor*.

When comparing variation in signal structure within regions, we found that both call types show little evidence of differentiation among the groups sampled in the Pacific region.

This result is not surprising, since the sites sampled represent demographically connected populations belonging to the same protected area. Here, geographic variation in vocalizations could result from stochastic processes such as drift and copying errors (Baker & Cunningham 1985; Podos & Warren 2007). A similar explanation can be attributed to the small levels of differentiation between LUM calls in the Caribbean sites, although these sites are currently isolated due to landscape fragmentation in the region (Sader & Joyce 1988; Fagan et al. 2013). However, variation of INQ calls do not show a gradual differentiation as would be expected if stochastic processes alone were the main driver in acoustic divergence in this call type. In summary, although we find evidence of acoustic differentiation of LUM calls among populations isolated by a historical barrier, a concordant pattern of geographic variation was not found for INQ. While divergence in call structure may reflect acoustic adaptation to distinct environmental conditions, the maintenance of call variants is likely to be better explained by social or genetic factors.

Patterns of acoustic divergence in relation to population genetic structure

Based on microsatellite distances, we found that genetic differentiation in populations of *T. tricolor* occurs both at a regional and local scale. However, the results of the clustering analysis show that population structuring does not correspond perfectly with the two sampled regions, as individuals from one site, Tapiria, were assigned to both the Pacific and Caribbean genetic clusters (Figure 3.5). This suggests that Tapiria represents a panmictic population and that gene flow is occurring despite the presence of a geographic barrier between regions. Although this result was unexpected, migration across the continental divide in lower Central America has been previously hypothesized to occur (Bagley & Johnson 2014). In Costa Rica, the low elevation areas at Cordillera de Tilarán have been suggested to be permeable to dispersal

across the continental divide in amphibians (Savage & Heyer 1967; Robertson & Zamudio 2009) and rodents (Demastes et al. 1996). Dispersal corridors at lower elevations may have facilitated gene flow in *T. tricolor*, influencing the lack of structuring between the Pacific populations and Tapiria field site.

In a setting of historical connectivity, gene flow would prevent divergence in a phenotypic trait, such as the vocalizations emitted by *T. tricolor*. While the patterns of acoustic variation in INQ calls strongly align with this lack of divergence (i.e., INQ call structure is similar among the Pacific populations and TAP field site), the geographic variation of LUM calls points towards a clear effect of the geographic barrier. Thus, contrary to our expectations, we found discordant patterns of acoustic variation for the two call types studied. While these calls are presumed to have similar functions in terms of facilitating associations between group members, our findings indicate that different mechanisms drive the process of signal divergence in INQ and LUM vocalizations. We suggest that LUM calls are socially transmitted vocalizations, while INQ calls are likely to be a genetically inherited trait.

Geographic variation of LUM signals indicates convergence of call structure within the regions sampled and divergence between regions. Although the variance partitioning results indicate that acoustic distance of this call type is primarily associated with genetic differentiation, which is itself influenced by geographic distance (Table 3.3), this pattern seems to be driven mostly by the genetic distance between Tortuguero, and the other 3 sites, including Tapiria. This pattern is supported by the analysis at a regional level, showing that this relationship is only present when considering the entire data set; neither spatial nor genetic variables explain the variation in LUM call structure *within* the Pacific or the Caribbean regions. Contemporary gene flow between the sites sampled within regions could be sufficient to

maintain homogeneity in the acoustic features of this call type. Vocal learning, a mode of social transmission, is a likely mechanism in a scenario of recent gene flow, as it could arguably occur faster than alteration of genetically inherited traits as a result of the multiple potential modes of social learning (Parker et al. 2012). Social learning can be transmitted vertically, from parents to offspring (e. g. evening bats, Scherrer & Wilkinson 1993; mouse lemurs, Kessler et al. 2014; marmots, Blumstein et al. 2015) and horizontally, between members of a social unit (e.g. meerkats, Townsend et al. 2011; parrots, Berg et al. 2012). In contrast, our data suggests that INQ calls are genetically inherited, as acoustic variation aligns with patterns of gene flow, as evidenced by the concordance found between genetic population structure and call divergence.

Despite the importance of multiple signal types in facilitating social interactions (Blumstein & Armitage 1997; Freeberg et al. 2012), the analysis of geographic variation on more than a single call type is surprisingly rare (Baker 2011). Although sparse, research documenting geographic variation of call types with different functions show that signals do not vary in the same pattern (Baker 2011), suggesting that the underlying selective forces shaping call design strongly influence the magnitude of call divergence. While the discordant pattern of geographic variation suggests distinct modes of transmission, this pattern also points towards different functions of these two vocalizations used by *T. tricolor*. The acoustic features of the two call types examined differ substantially; INQ calls are short broadband signals that are most likely involved in locating group members at a closer range compared to the narrowband multi-harmonic structure of LUM calls. From a signal design perspective LUM calls have been suggested to be involved in long-distance communication (BKM & EHG, unpublished data). Furthermore, while LUM calls seem to be exclusively emitted during night activity, INQ calls are not only the prevalent signal type recorded during the night (BKM & EHG, unpublished

data), but also enable bats to regroup after a disturbance event during the day (Chaverri et al. 2010). The different contexts in which these two calls are observed to occur suggest distinct mechanisms of social selection on these calls, despite clearly having an affiliative function in *T. tricolor*. Although previous work has shown that INQ calls encode information about individual identity (Gillam & Chaverri 2012); determining whether LUM calls show individual or group signatures is particularly challenging, as bats do not emit LUM calls under an experimental setup and individual identity of free-ranging bats is logistically challenging. Future work addressing this issue would provide great insight with respect to the plausibility of this signal being a socially learned trait.

Despite the central role of acoustic communication enabling social interactions, studies addressing geographic variation of social calls in bats are rare. Most work has focused on elucidating the patterns of variation in echolocation signals (Puechmaille et al. 2011; Jiang et al. 2010; Fornůsková et al. 2014); while echolocation calls can potentially have a communicative function (Voigt-Heucke et al. 2010; Knornschild et al. 2012), they are ecologically and functionally constrained by the physical environment in which bats are found (Schnitzler & Kalko 2001). Here, we expand our current knowledge of geographic variation of vocalizations emitted by bats. Further research is needed to obtain a comprehensive picture of the role of vocal learning and genetic transmission of contact calls emitted by *T. tricolor*.

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IV. SPACE USE BY A LEAF-ROOSTING BAT

Introduction

Living in groups (i.e. sociality) has numerous benefits, such as reduced predation risk (Hill & Dunbar 1998), increased access to resources (Yip et al. 2008; Cameron et al 2009) and thermoregulatory advantages (Ancel et al. 1997). Yet, group living is also characterized by conflicting interactions (e.g. competition, Wrangham 1980) that can negatively impact individuals. Such cost-benefit dynamics, which typically are driven by the fluctuating nature of the local environment, can directly impact the adaptive nature of different spatiotemporal patterns of group association (Peres 1989). Within the field of behavioral ecology, this socio-ecological framework is used to describe and classify animal social systems (Koenig et al. 2013). Social structure, a component of social systems, is defined by the quality and patterns of interactions (Hinde 1976). Unfortunately, there is a tendency for researchers to place species into broad categories of social structure based on short periods of observation; this is problematic, as such a sampling scheme is unlikely to capture natural variation in the system, particularly in response to changing levels of resource availability and/or environmental conditions. Arguably, the description of social structure should be viewed as a dynamic process, whereby the patterns of group formation fall within a continuum of social options.

An additional problem with standard methods of assessing social structure is that data is generally only gathered during part of the 24 hour daily cycle. Describing and classifying social structure requires knowledge of detailed information about interactions between group members, which is typically a very challenging task. This is particularly true for highly mobile species, as tracking individuals is typically restricted by logistics to small sections of an individual's home range and limited to specific time periods. As a result, there is a tendency to only assess patterns

of association during the time period when animals are stationary or resting. This restrictive pattern of sampling is pervasive in studies of social structure for most mammalian groups, with the exception of primate species and a small number of cetaceans (Rendell & Whitehead 2001; Clutton-Brock & Janson 2012). The difficulty in studying mammalian behavior is also linked to nocturnal habits, as nearly 70% of mammalian species are nocturnal (Bennie et al. 2012).

As a group, bats can be particularly difficult to track during the entire 24 hour cycle. Bats fly rapidly across the landscape, with some species travelling as far as 50km away to forage in a single night (e.g. free tailed bats, Marques et al. 2004; Rhodes & Catterall 2008). Until recently, group interactions among bats were quantified by directly observing individuals (e.g. Goodall 1963). Standard radio-telemetry data has been very successful for characterizing associations among individuals during the daytime roosting period (Kerth et al. 2001; Campbell et al. 2006; Rhodes 2007; Popa-Lisseanu et al. 2008), but has been of limited use for tracking associations at night when bats are actively foraging across the landscape. However, the development of automated tracking devices have expanded our ability to study otherwise cryptic animal systems ((e.g. sharks, Voegeli et al. 2001; bats, McGuire & Guglielmo 2012), offering the opportunity to gain a better understanding of the behavioral strategies facilitating group formation in bats.

This study examines the patterns of group association of the Neotropical disc-winged bat, *T. tricolor*. Bats use discs located on the base of the thumbs and ankles to attach to the surface of furled developing leaves of musoid plants (Wilson 2008). Given the morphological specialization of disc-winged bats, individuals must cope with the ephemeral availability of furled leaves, which forces bats to continuously search and locate suitable roosts (Findley & Wilson 1974; Vonhof & Fenton 1999). *T. tricolor* forms mixed-sex groups that remain loyal to

small patches of forest (~0.4 ha). Previous work based on capture-recapture studies determined that individuals within a roosting group form long-lasting associations, suggesting a highly cohesive social system (Chaverri 2010). Here, we use an automated radio-telemetry array to expand our knowledge about the social system of this species by characterizing group interactions of free-ranging bats throughout the night.

Our main objective was to study the movement patterns of *T. tricolor* during the night. The range of the telemetry system covered most or all of a group's roosting range, allowing us to determine to what extent the movement patterns of bats during foraging bouts were restricted to their roosting range. We hypothesize that bats spend the majority of their time within the bounds of their roosting home range. We also aimed to quantify the frequency of encounter rates among bats in a group during the monitoring period. Given that *T. tricolor* needs to locate new roosts every night, examining patterns of group member interactions offers important insight into the behavioral strategies involved in coping with the use of an ephemeral roosting resource. Based upon our knowledge of their patterns of association in roosts (highly stable), we hypothesize that bats will also exhibit high levels of association on the wing at night.

Methods

Study species

Spix's disc winged bat, *Thyroptera tricolor*, is a small (3-4 g) insectivorous bat inhabiting humid lowland forests ranging from southern Mexico to southern Brazil (Wilson 2008). The species is characterized by the presence of discs on thumbs and feet that enable individuals to cling to the smooth surface of furled leaves of developing musoid plants. Furled leaves remain suitable for roosting during very short periods of time (~8 -90 h), forcing bats to locate new roosts daily (Vonhof & Fenton 1999). Bats are primarily found along streams and

forests clearings where stands of *Heliconia* and *Calathea* plants are commonly distributed.

Features of *T. tricolor*'s echolocation and morphology suggests that bats use a gleaning foraging strategy to capture prey (Norberg & Rayner 1987; Fenton et al. 1999), feeding on arthropods found on the surface of vegetation (Dechmann et al. 2006). Capture data suggests that roosting groups show high spatial fidelity (Vonhof et al. 2004; Chaverri & Kunz 2011) within relatively small home ranges (> 1 ha), however information about patterns of nightly activity and association are unknown.

Study site

The study was conducted during the months of June and July of 2011 and 2012 at San Pedrillo field station (8.62° N, -83.73° W), within Corcovado National Park, a protected area located in southeastern Costa Rica. Field work was done in lowland forest where the terrain is relatively flat, with numerous streams draining throughout the study area. The habitat in which groups of *T. tricolor* were studied is characterized by moderately dense understory cover where patches of *Heliconia* spp. and *Calathea* spp. are abundant. Large tree species are interspersed, creating a canopy that is over 25 m high (Herwitz 1981). The site receives an average of 475 mm of precipitation during the rainy season (from May to November) and 130 mm during the dry season (from December to April). We collected data during the early wet season, corresponding to the post-lactating reproductive period of *T. tricolor*.

Sampling of movement patterns

Bats were captured during the day at roosting sites by pinching the top of the leaf and directing individuals into a cloth holding bag. We recorded standard measurements (body mass, forearm length, sex, reproductive state and age) of all the individuals captured. Individuals were fitted with coded transmitters (Lotek NTQ-1 Nano tags, 5s pulse rate; Lotek Wireless). We

trimmed a small section of the dorsal fur between the scapulae to attach the transmitter using skin bond cement (Ostobond, Montreal Ostomy). Transmitters weigh 0.25g, including an 18cm flexible antenna that did not interfere with the roosting behavior of bats within a leaf. Tags fell off or were removed within 10 days after being affixed to bats. No signs of injury were observed after tag removal. The process of taking measurements and attaching the radio tags to all bats in a focal group lasted approximately 40 minutes. Bats were released simultaneously, which typically allowed individuals to regroup in close proximity to the previous day roost. Video recordings for another study made in the vicinity of furled leaves showed that tagged bats continued to display normal flight behavior.

To assess the movement patterns of individuals within a roosting group, we used an automated digital radio-telemetry array that allowed us to simultaneously monitor the movement of tagged bats during the night. A unique digital signature is associated with each transmitter, allowing for individual identification while only monitoring one frequency. The telemetry array was composed of two 5-element Yagi directional antennas and a data-logging receiver (SRX DL, Lotek Wireless). We recorded the geographic location of the focal group for at least 3 days prior to tagging to obtain an estimate of the roosting home range. This enabled us to identify a suitable position for mounting the radio-telemetry array that was near the center of the group's home range. Two arrays were positioned at ~ 50 m from each other (Figure 4.1a). The antennas were mounted on trees at a height of approximately 5 m, oriented in opposite directions and aligned to the second array of antennas. The receiver gain was set at 80 dB, which allowed us to limit detection range of the receiver to the group's roosting home range and the immediate surrounding areas. The automated scan cycle of each receiver was set to scan for each radio frequency every 6 s, sequentially on the first antenna before switching to the second antenna.

Once a frequency was detected, the receiver was set to immediately switch to the next frequency listed. The maximum scan time per antenna of a focal group consisted of 5 tagged individuals, corresponding to 30 s, but could be less if bats were all within range and sequentially detected. When a transmitter was detected, the receiver recorded the ID code of the transmitter, time, antenna (1 or 2), and signal strength.

Calibration tests

Automated telemetry devices permit the user to define a variety of settings, allowing the system to be optimized in reference to the objectives of a study. Given this flexibility, calibration tests are essential to determine the criteria that need to be taken into consideration during data analysis so that errors associated with the limitations of the system can be minimized. We conducted two distinct calibration procedures: First, we estimated the correspondence between signal strength and distance with respect to vegetation density and landscape characteristics at each site. To make this estimation, we started at the location of each receiver and walked 4 straight-line transects at 90° angles from each other (Appendix D). Along each transect, we recorded signal strength for 60 s at fixed intervals of 10 m from the receiver until signal strength attenuated to 0. The points at which measurements of signal strength were taken along each transect were georeferenced using a handheld GPS. Second, we determined the variation of signal strength in relation to the proximity distance between two tags. To estimate such joint detections, we recorded signal strength of two tags positioned at 1, 2, 3, 4, 5 and 10 m from each other, along 4 straight-line transects, repeating the measurements at 10 m fixed intervals. This was repeated at two locations. We tested for a relationship between difference in signal strength and intraspecific tag distance using a linear mixed effect model. Tag proximity was fitted as a categorical fixed effect. Because we conducted repeated measures of intraspecific signal strength

difference along the 10 m distance intervals from the receiver (i.e. distance from the receiver) at two locations, 'distance from receiver' and 'location' were fitted as nested random effects. Both calibration approaches simulate static animals at a pre-set distance from the telemetry array. To mimic the detection conditions of tags attached to bats, we used clay models of approximately the same size of *T. tricolor* and secured the tag to the dorsum of the model. Clay models were attached to a string and suspended at 2 m from the ground level (Appendix D).

We used the geostatistical approach, commonly known as Kriging, to describe the patterns of signal strength with respect to distance. The values recorded at each point of the validation transects were averaged and then used as point measurements to create maps that interpolate these data over the detection range of the telemetry array. We created prediction maps using the Universal Kriging model applied on ESRI's ArcGIS Geostatistical Analysis Software (Fig. 4.1b). These maps were then used to estimate the detection area covered by the telemetry array for each group monitored. In addition, during the daytime we searched for the tagged group using real time monitoring of the SRL-DX receiver attached to an antenna and displaying data through the computer. When a roost was located, we recorded the position (GPS coordinates) and visually confirmed the presence of group members. The locations of day roosts were then used to estimate the roosting home range (computed as the minimum convex polygon-MCP) of a group during the length of the study (Figure 4.1a).

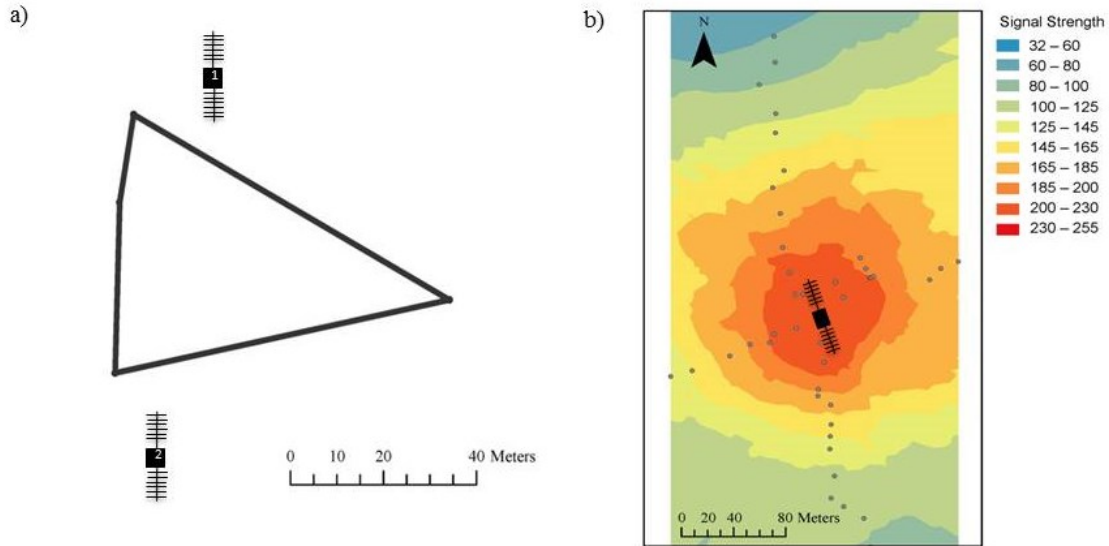


Figure 4.1. Example of the telemetry setup and detection range for group 29. a) Position of the two telemetry arrays (illustrated as a receiver and the two antennas) with respect the estimated roosting home range of group 29. b) Interpolation map depicting the variation in signal strength with respect distance from the receiver. Points indicate the position of each 10 m distance interval along the 4 transects.

Data analyses

Since data is collected at irregular time intervals between individual tag records after each scan cycle, we computed a running mean incorporating records within a 10 s window, adjusting the potential lag between consecutive detections. A focal group was monitored for 5 to 9 continuous nights; to create equal sample sizes for analyses, we randomly selected 5 nights across groups. Data management and analyses were conducted using R (R developing team, 2015).

Inference of movement patterns.- Because the detection range of the telemetry system included the roosting range of the focal group as well as up to 2.2 km² surrounding areas, we were able to infer the extent to which an individual was detected within the estimated area covered by the telemetry array. For each bat, we first tabulated detections lasting at least 30 s. This allowed us to filter out spurious detections logged from individuals moving close to the

limits of the detection range. We then measured the duration of continuous detections and estimated the total length of time that a radio-tagged bat was detected throughout the night. Detections were quantified from 18:00-05:30, corresponding to the times in which bats were previously observed to leave and enter a day roost, respectively. Based on this monitoring time span, the total amount of time a bat could be detected if it continuously remained within the detection range of the telemetry system was 41,400 s per SRX-DL receiver. We estimated the proportion of time a bat was detected at both receivers using this maximum detection time for each night monitored. For instance, records for bat T102 indicated that the bat spent a total duration of 37950 s within the detection range of the two receivers on ‘day 1’; this value was divided by the maximum possible time a tag could be detected (82800 s considering both receivers). Bat T102 was estimated to spend 46% of the night within the detection range of the receivers.

To assess whether there was a difference between males and females in the proportion of time a bat was detected within range of the telemetry receiver, we used a generalized linear mixed model (GLMM) with a binomial logit link function (Zuur et al. 2009). Since data was collected repeatedly for each bat across 5 nights, we fitted night as a random effect. To investigate the similarity in the proportion of time bats within a group were detected across days (i.e. whether all bats within a group would be detected similar amounts of time across nights), we used the nonparametric Kendall’s coefficient of concordance (W). This value ranges from 0 to 1, 1 being high concordance in the proportion of time group members spent within the receiver’s detection range. The global Kendall W statistic and associated P values derived from 10,000 permutations were estimated using the *vegan* package in R.

Quantifying dyads. - We quantified the occurrence of a dyad (i.e., when two bats were detected within close proximity) based on a set of rules for estimating joint detections. Bats were considered to be in close proximity if: 1) the signal strength difference between records was equal or less than 35, and 2) this difference lasted at least 30 s. This cut-off threshold of signal strength was derived from the validation transects in which we determined that on average, tags 1 m apart exhibited a signal strength difference of 33.5 (Figure 4.2). We tabulated the start and end time of each dyad and measured the length of a joint detection in seconds. Joint detections greater than 1 min apart were considered separate events. To describe the patterns of joint detections across time, we grouped observations in 30 min time categories from 18:00-05:30. To test for differences in the frequency of dyadic interactions between groups and time categories, we used a GLMM with a Poisson distribution appropriate for count data. Night was fitted as a random effect. To correct for the fact that each bat spent a different period of time within the detection range of the receivers, we normalized dyad duration in reference to the amount of time that *both* bats were within receiver detection range for a given time category. We generated heat maps depicting the proportion of time that two bats spent in close proximity across the night. These maps allowed us to visually describe the patterns of close proximity for each dyad within and between nights. Values close to 1 indicate that for a given time category, a pair of bats spent most of their time within close proximity when both individuals were within the detection range of either receiver.

Results

We monitored 23 bats belonging to 4 groups during the period of the study. The sampling of movement patterns using an automated telemetry system resulted in a total of 863,324 tag detections across the four groups. The validation of the telemetry system revealed that while

signal strength decreased with respect to distance from the receiver ($F_{1,116}=82.53$, $P<0.001$, $r^2=0.54$), there was extensive variation between sites ($F_{3,116}=14.44$, $P<0.001$). This variation most likely results from differences in vegetation structure between sites. Based on the interpolation maps, we estimated that the detection range of the receivers covered an area of 2.2 (± 0.3) km² which included the roosting range of all groups. We found extensive variation in the proportion of time each bat was recorded within the range of the telemetry system (Table 4.1). When examining whether sex influenced the length of time bats were detected, we found that males significantly spent less time within the area compared to females (GLMM, z -value = -3.21, $P=0.001$).

The analysis of level of concordance in time spent within the roosting range and immediate surrounding areas differed between the groups studied. We found a significant level of concordance in the proportion of time bats within a group spent within the detection range of the telemetry system for two of the four groups monitored (Table 4.2).

Table 4.1. Proportion of time each tagged bat was recorded within the detection range of both devices.

Group	Bat ID	sex	Receiver 1					Receiver 2				
			1	2	3	4	5	1	2	3	4	5
23	T109	f	0.61	0.08	0.47	0.91	0.65	0.85	0.29	0.56	0.94	0.95
	T110	m	0.78	0.49	0.57	0.28	0.00	0.44	0.27	0.00	0.00	0.00
	T111	m	0.62	0.29	0.80	0.83	0.28	0.70	0.52	0.85	0.98	0.96
	T112	f	0.04	0.13	0.30	0.24	0.48	0.06	0.00	0.08	0.00	0.51
	T113	f	0.76	0.32	0.89	0.79	0.27	0.66	0.00	0.33	0.05	0.04
26	T114	f	0.91	0.90	0.69	0.87	0.77	0.55	0.59	0.64	0.60	0.47
	T115	m	0.73	0.73	0.27	0.42	0.21	0.29	0.42	0.26	0.20	0.20
	T116	f	0.89	0.84	0.30	0.30	0.73	0.20	0.31	0.17	0.19	0.43
	T117	f	0.71	0.68	0.71	0.09	0.68	0.13	0.33	0.50	0.04	0.25
	T118	f	0.66	0.78	0.76	0.22	0.32	0.13	0.49	0.45	0.14	0.21
29	T102	m	0.66	0.01	0.70	0.37	0.61	0.26	0.78	0.77	0.60	0.65
	T108	m	0.71	0.01	0.56	0.37	0.50	0.26	0.73	0.54	0.52	0.58
	T115	m	0.51	0.01	0.80	0.33	0.19	0.33	0.69	0.78	0.63	0.30
	T116	m	0.78	0.00	0.87	0.51	0.51	0.20	0.71	0.78	0.78	0.50
	T117	f	0.86	0.00	0.87	0.49	0.64	0.14	0.50	0.80	0.85	0.61
	T118	m	0.82	0.00	0.77	0.36	0.41	0.21	0.34	0.66	0.51	0.52
44	T124	m	0.09	0.07	0.07	0.06	0.38	0.11	0.12	0.50	0.00	0.73
	T125	m	0.15	0.12	0.08	0.10	0.66	0.14	0.58	0.65	0.51	1.00
	T128	m	0.19	0.24	0.18	0.11	0.42	0.09	0.46	0.60	0.00	1.00
	T129	m	0.27	0.20	0.12	0.14	0.51	0.21	0.15	0.51	0.21	1.00
	T130	m	0.07	0.11	0.04	0.09	0.60	0.11	0.62	0.55	0.62	1.00
	T131	m	0.22	0.12	0.09	0.05	0.45	0.15	0.46	0.39	0.09	1.00
	T134	f	0.16	0.21	0.21	0.13	0.63	0.10	0.65	0.46	0.71	1.00

Table 4.2. Information about group size, roosting range and results of concordance analyses for four groups monitored using an automated telemetry system across a 5 night period. Kendall’s coefficient of concordance (W) and associated P values indicate whether bats within a group showed a concordant pattern in the proportion of time spent within the detection range of the telemetry system.

Group ID	Group Size	MCP	Kendall W	P
G23	5	0.25	0.184	0.479
G26	5	0.59	0.360	0.118
G29	6	0.19	0.750	<0.001
G44	7	0.11	0.465	0.008

We defined a joint detection based on the validation procedure in the field over which two tags were tested to mimic 1 m distance between each other. These tests resulted in an average of 33.5 difference in signal strength, and a value of 35 was used as the threshold to filter the data for records deemed as a joint detection (Figure 4.2). The mean difference in signal strength varied significantly across the 6 tag distances ($F=3.08$, $P=0.014$).

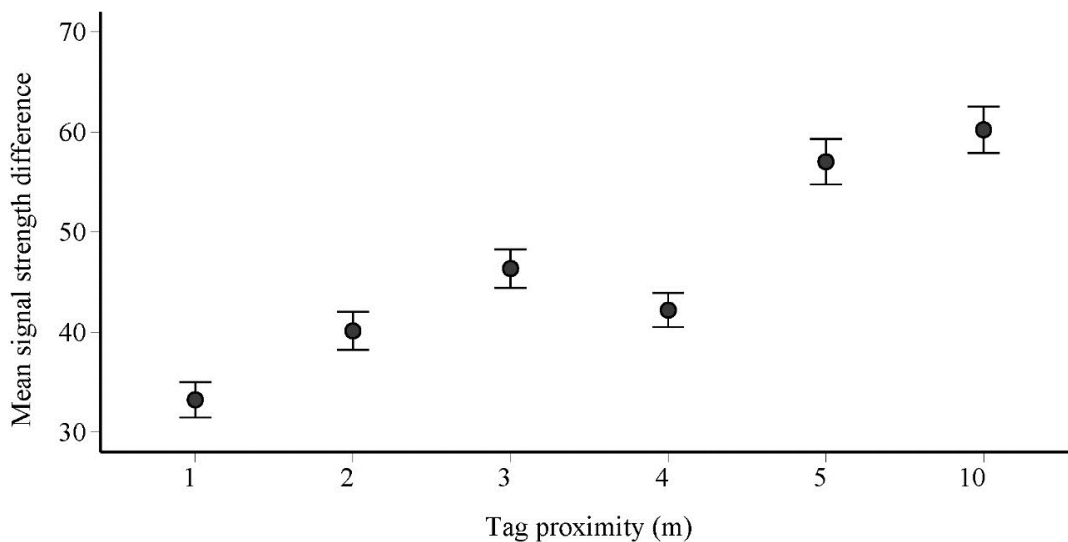


Figure 4.2. Mean difference in signal strength between two tags positioned at 1,2,3,4,5 and 10 m apart from each other.

Our analyses of joint detections revealed extensive variation with respect to the frequency and duration of dyad detections. We determined significant differences in the mean frequency of dyad events across the night between all groups studied (Figure 4.3), with G44 exhibiting the highest mean frequency of dyadic detections (GLMM, z -value=11.94, $P < 0.001$). The frequency of dyads remained relatively similar throughout the night; we found no significant differences between the mean frequency of dyads recorded at each 30 min window interval.

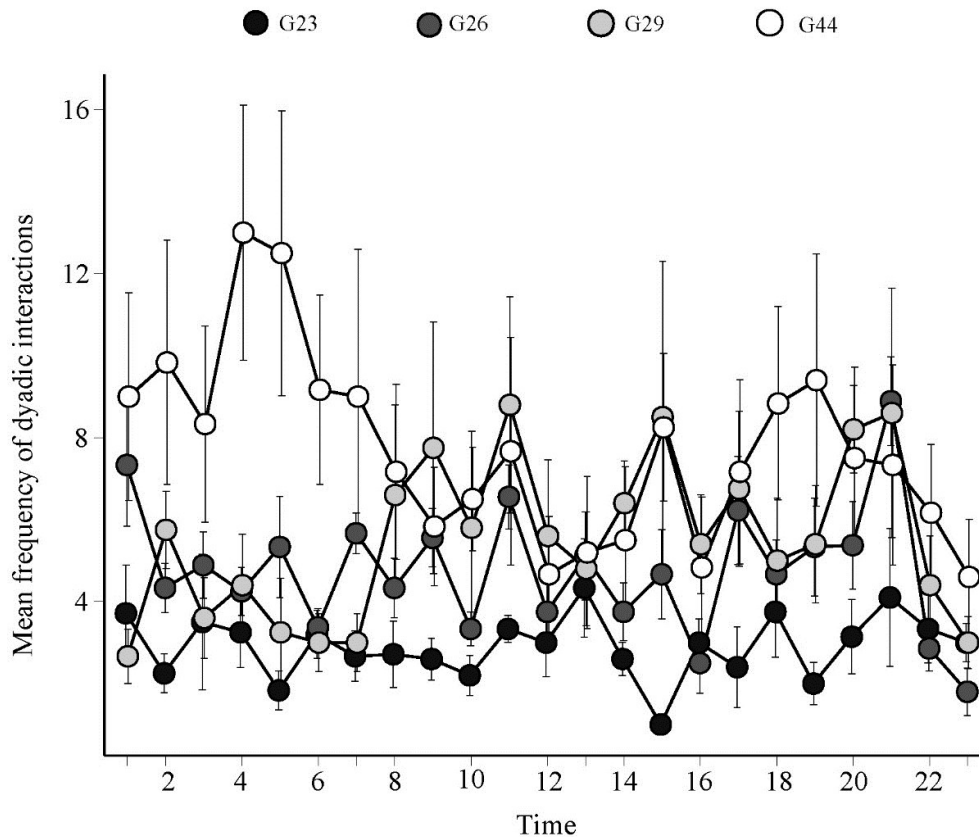


Figure 4.3. Mean frequency of dyadic records detected during continuous monitoring of radio tagged bats belonging to 4 groups during the night. Observations were grouped in 30 min time categories. The different shades represent each group studied.

The duration of joint detections ranged from 20 – 1629 s, with an overall average of 190.4 (± 4.90) s. When two bats were identified as being in close proximity, we estimated the length of time the dyadic interaction occurred with respect to the total time these two bats were within the detection range. If multiple events (defined by joint detections separated by more than 60s), we computed the total time a dyad remained in close proximity for a 30 min window. This information was tabulated as the proportion of time recorded for a given dyad. Close to 92% of these events corresponded to relatively short interactions (≤ 10 min). Dyads lasting more than 15 min were rare, but were observed to occur throughout the night and across all groups. An examination of the heat maps generated to describe the temporal patterns of joint detections during a 5 night sampling period revealed that a given pair of bats encountered each other repeatedly during the night. Furthermore, encounters do not appear to follow a clear temporal pattern, suggesting that group members are regularly in close proximity, independent of the time of night.

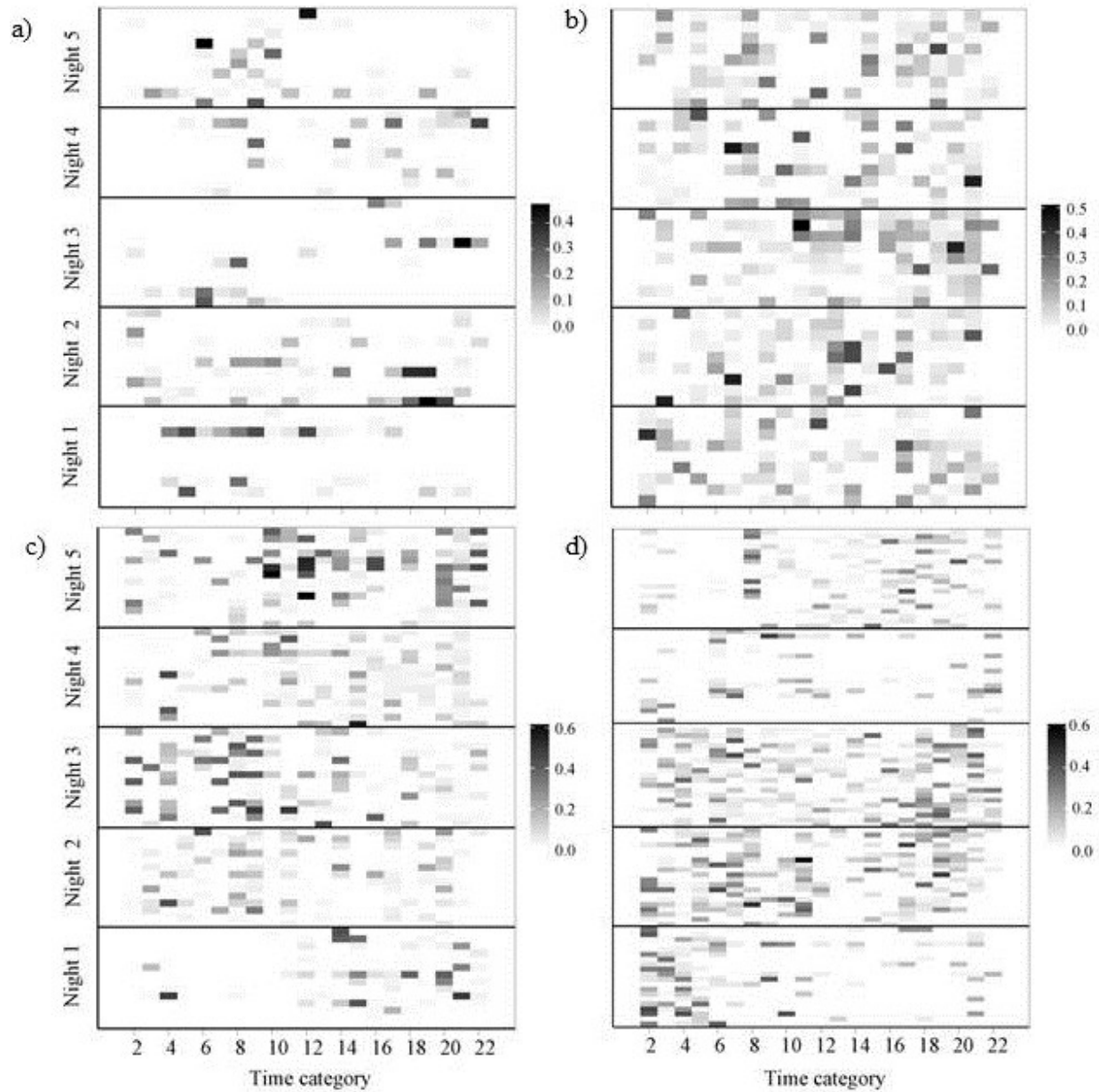


Figure 4.4. Heat maps depicting the proportion of time a given pair of bats was estimated to be in close proximity during the night for a period of 5 sampling nights. Observations were grouped in 30 min time categories represented by columns. Rows correspond to a dyad. a) Group 23, b) Group 26, c) Group 29 and d) Group 44.

Discussion

The primary focus of this study was to characterize the dynamics of space use by *T. tricolor*, a bat species that exhibits a highly cohesive social structure despite the need to regularly switch roosts. Using an automated telemetry array, we were able to continuously monitor multiple individuals within a social group. This approach enabled us to first, assess whether bats remain within the area delimited by their roosting range, and second, determine the frequency of encounters between group members.

The proportion of time bats were detected within range of the telemetry system varied extensively between bats, throughout the night and across the five day period. A lack of consistency with respect to space use is not surprising given that foraging bats are likely to actively respond to the distribution of prey. *T. tricolor*'s diet consists of arthropods found on the surface of vegetation, and analysis of fecal samples identified wandering spiders as *T. tricolor*'s primary prey (Dechmann et al. 2006). Studies addressing the spatial distribution of wandering spiders in tropical forests suggest that while patterns of abundance differ between species, there is little evidence of clumped distributions; rather spiders tend to be found uniformly throughout the forest, responding to microhabitat characteristics such as soil and vegetation structure (Gasnier & Hofer 2001; Souza & Martins 2005). Therefore, to meet their energetic demands, bats likely spend at least part of the night searching for prey outside of the roosting area; how far away bats travel from their home range remains to be explored. Although some individuals spent relatively short lengths of time within detection range for a given focal time window (30 min), it is unlikely that individuals traveled extensive distances. First, *T. tricolor*'s wing morphology is well suited for maneuverability within the dense forest interior, but poorly suited for achieving high flight speeds (Norberg & Rayner 1987). Second, the longest period of time a bat was out of

range was 40 min, suggesting that even when bats were not being detected by the telemetry array, they were not far away.

The analysis of concordance indicates that two out of the four study groups exhibited significant levels of concordance (47 to 75 %) in the proportion of time that all group members were detected within range. This result suggests that bats spent a similar proportion of their time within the detection area indicating a high level of group cohesion. The contrasting results between groups could result from differences in the long-term association patterns of each group. While previous research suggests that *T. tricolor* forms stable group associations, this assessment is based on monthly censuses (Chaverri 2010), which may not reveal more short term (i.e. nightly) changes in group composition. For example, during the 10 days we sampled roosting group G23, two bats (T109 and T110) each roosted away from the group for a single night (although, not the same night). Differences in the telemetry setup could also partially influence our findings, although for all groups, the setup of the telemetry array was arranged in a way that the entirety of a group's roosting range was within the system's detection range.

Groups of *T. tricolor* consist primarily of matrilineal relatives characterized by all offspring philopatry (Chaverri & Kunz 2011). While the behavioral mating strategies of *T. tricolor* remain unknown, recent work has shown that reproduction occurs between unrelated individuals, suggesting that bats travel relatively long distances to mate (Buchalski et al 2014). We found significant differences in the patterns of space use between females and males. Females remained within detection range for longer periods of time compared to males. An exploratory behavior in males could represent a selective advantage by increasing the likelihood of mating success. Collection of additional data that examines patterns of association during and outside of the mating season would be valuable for better understanding how reproductive

behaviors impact group cohesion both during nightly foraging bouts and within daytime roosting groups.

On a given night, *T. tricolor* must invest time in foraging as well as locating a day roost. Our results provide evidence that group members fly in close proximity for short bouts of time (most encounters lasted >10 min) throughout the entirety of the night. While this pattern could simply reflect overlap in the foraging areas used by different bats, this seems unlikely given that we censored joint detections to represent a distance of ~ 1 m between flying bats; this means that we excluded detections that could still reflect bats at relatively close distances (~2-5 m apart). Hence, our approach is conservative, since interactions, such as information exchange through acoustic signals, could still occur at greater distances. Our evidence for consistent social interaction throughout the night agrees with data from video recordings made in the vicinity of furred leaves for another study (Montero and Gillam, in press), in which multiple bats performed a chase behavior.

Joint detections lasting more than 10 min were rarely observed, although they did occur repeatedly during each night. These long lasting encounters could represent two or more bats remaining on the wing together for extended periods of time, or it could be represent multiple bats sharing the same night roost. Montero and Gillam (in press) found that use of night roosts by *T. tricolor* is very common, although it is not clear how often these sites are shared by more than one bat. Gillam and Montero (in press), also documented a number of distinct social calls emitted when bats are flying near furred leaves; such contact calling is likely pivotal in allowing individuals to regroup after being separated for periods of time throughout the night.

The nocturnal movement patterns described here are congruent with behaviors observed in species with fission-fusion dynamics (eg. tamarin monkeys, Garber et al. 1993; lemurs,

Kappeler 1997; bottlenose dolphins, Lopez & Shirai 2006). Within a group's roosting range, *T. tricolor* appears to forage in subgroups. These associations might aid in territorial defense (Connor & Whitehead 2005), as observed in hyenas (Boydston et al. 2003), and spider monkeys (Wallace 2008). One potential benefit of forming stable group associations in *T. tricolor* might be the ability to monopolize patches of habitat that support a regular supply of furred leaves, selecting for a territorial behavior. Regular encounters also facilitate information transfer (Kerth et al. 2006; Guttal & Couzin 2010, Lasseau 2007). In *T. tricolor*, the process of roost selection is likely a process of group decision making, although future work is necessary to identify how this process takes place. The regular dyadic interactions throughout the night observed in this study could be the periods of time when information transfer about the location and suitability of available furred leaves occurs between group members.

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V. CONCLUSIONS

The need of organisms to use resources that fluctuate in quality and availability over time and space may result in important selective pressures driving the evolution of animal social systems. Thus, examining the influence of ecological constraints on social behavior is critical for understanding the adaptive significance of sociality. This dissertation aims to provide a comprehensive picture of how the ecological pressures associated with roosting in leaves have shaped the evolution of behavioral strategies used by Spix disc-winged bats, *Thyroptera tricolor*. These bats have developed highly specialized morphological adaptations that enable individuals to roost inside furled musoid leaves. Use of such an ephemeral roosting resource presents major challenges, as leaves rapidly unfurl, forcing animals to locate new roosts on a daily basis. Despite the reliance of *T. tricolor* on such an ephemeral roosting resource, bats form long-lasting stable social groups composed primarily of close kin (Chaverri 2010; Buchalski et al. 2014).

The need of *T. tricolor* to relocate new roosts on a daily basis led us to hypothesize that bats visit furled leaves within a groups' roosting range to assess its suitability as a day roost. We determined that bats regularly monitor furled leaves within their roosting range, during which they emit a number of distinct social calls. Roost assessment involved close inspection of leaf dimensions and surroundings, and was mostly performed by single individuals. *T. tricolor*'s call repertoire during flight indicates that group contact involves a combination of acoustic signals. This study used a non-invasive approach, which allowed us to assess the behavior of bats without disturbing local conditions, such as the availability of suitable leaves. Although I was able to characterize the behavior of bats evaluating roost suitability, important questions remain to be explored, in particular the process of roost selection.

Studies addressing geographic variation in vocalizations produced by mammals are sparse. Although patterns of vocal divergence have been identified in a variety of species (e.g. sperm whales, Rendell & Whitehead 2003; seals, van Parijs et al. 2003; orangutans, Delgado 2007; pikas, Trefry & Hik 2010), the sources of geographic variation in mammals are still not well understood. In bats, social calls are pivotal in facilitating a variety of interactions (Gillam and Fenton, *in press*), however almost all attention to on geographic variation in acoustic signals produced by bats has been given to echolocation calls (Puechmaille et al. 2011). I expand on our current knowledge of geographic variation of social vocalizations emitted by bats by studying the geographic variation in microsatellite allele frequencies at multiple sites on the Caribbean and Pacific mountain slopes.

The behavioral strategies used by *T. tricolor* near furled leaves indicates that group behaviors, such as coordination and information exchange among group members, are likely mediated by a variety of acoustic signals known as contact calls. Divergence in calls emitted by *T. tricolor* could be driven by environmental, genetic or social factors. I examined whether the patterns of acoustic variation in two contact calls regularly emitted by Spix's disc winged bats, *Thyoptera tricolor*, are congruent with patterns of genetic distance among populations isolated by a geographic barrier. Comparing multiple sites within each region allowed me to test specific predictions with respect to patterns of genetic and acoustic differentiation. The roosting ecology and grouping behavior in *T. tricolor* led me to hypothesize that while a gradual pattern of acoustic differentiation would be present within regions due to stochastic processes, limited gene flow between populations separated by the geographic barrier would result in call divergence that aligns with the patterns of population genetic structure. I found that the patterns of geographic variation differ between the two types of calls studied. I argue that this difference

results from the distinct modes of vocal transmission within populations. My results suggest that while one of the contact calls examined is likely to be socially transmitted through processes of vocal learning, the congruence between the patterns of genetic differentiation and acoustic variation between the second type of contact call is likely to be an inherited trait.

The specialized roosting ecology of *T. tricolor*, coupled with the cohesive social structure observed in roosting groups suggests that individuals regroup after periods of separation at some point during the night. I used an automated telemetry receiver to continuously monitor the movement patterns of bats within an area that included the roosting range of a group. Results suggest that bats are mainly foraging within the roosting group's daytime roosting area. Nocturnal activity is also characterized by the presence of regular dyadic encounters of variable length. These patterns of space use could allow bats to both monitor the suitability of furred leaves as well as monopolize a patch of habitat that offers a regular supply of furred leaves, an essential resource for *T. tricolor*.

This study provides a comprehensive picture of how ecological pressures associated with ephemeral roosting resources have impacted the behavioral strategies and population dynamics of a highly specialized bat. Furthermore, the findings documented here will serve as a baseline for future research concerning the process of decision-making in a species of bat that confronts the need to select, potentially as a group unit, a vital resource on a daily basis.

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APPENDIX A. INFORMATION ABOUT SAMPLED SOCIAL GROUPS AT EACH SITE

Site	Group ID	Group size	MCP	N _{focal leaves}	N _{night roosts}	Acoustic recording (h)
SIR	1	4	0.6889	2	1	15.73
	4	5	0.6542	3	1	22.29
	6	4	0.3623	3	2	25.02
	7	5	0.773	2	1*	16.84
	10	2	0.2111	2	1	13.16
	11	5	0.4534	2	-	9.83
TOR	30	4	0.12115	2	-	11.56
	34	9	0.499	2	-	12.05
	36	5	0.26365	3	1	25.65
	39	5	0.7469	4	-	30.47
	40	6	0.2012	3	-	21.62
TAP	41	6	0.1448	3	-	34.7
	42	6	0.3497	4	2*	35.26
	43	3	0.1364	3	1	22.76

**APPENDIX B. PRINCIPAL COMPONENT LOADINGS FOR THE RETAINED AXES
FOR INQ AND LUM CALLS OF *T. TRICOLOR***

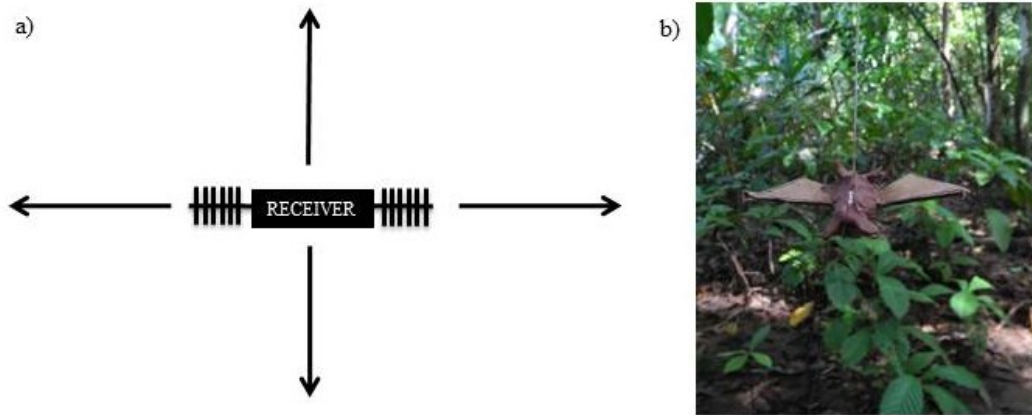
Call parameter	INQ				LUM		
	PC1	PC2	PC3	PC4	PC1	PC2	PC3
dur_c	-0.258	0.133	-0.135	0.417	-0.050	0.278	0.245
f_{peak}	0.064	0.399	-0.083	-0.060	0.286	-0.042	-0.131
f_{start}	0.075	0.406	-0.088	-0.091	0.327	-0.069	-0.206
f_{end}	-0.117	-0.063	0.529	0.085	0.096	0.117	0.250
f_{max}	-0.013	0.274	-0.009	-0.014	0.108	0.098	0.219
f_{min}	-0.091	-0.046	0.528	-0.005	0.317	-0.064	-0.191
S_{min}	-0.272	-0.112	0.176	-0.019	-0.036	0.238	0.082
S_{max}	-0.048	-0.071	0.003	0.488	-0.104	0.046	0.428
$S_{95 \text{ percentile}}$	-0.116	-0.038	0.054	0.544	-0.127	0.008	0.406
C_{min}	-0.237	-0.021	0.087	-0.061	-0.022	0.224	-0.014
C_{min}	0.254	0.004	-0.066	-0.005	-0.039	-0.203	0.0812
$C_{95 \text{ percentile}}$	0.251	0.022	0.006	-0.095	0.011	-0.246	-0.041
Variance	0.32	0.21	0.17	0.14	0.32	0.31	0.20

**APPENDIX C. MEASURES OF GENETIC DIVERSITY OF 10 MICROSATELLITE
LOCI USED TO ESTIMATE GENETIC POPULATION DIFFERENTIATION IN *T.***

TRICOLOR

Locus	Sirena			San Pedrillo			Tortuguero			Tapiria		
	N	Ho	He	N	Ho	He	N	Ho	He	N	Ho	He
Tt2	10	0.54	0.82*	11	0.74	0.81	11	0.82	0.84	8	0.64	0.87
Tt4	5	0.35	0.38	2	0.26	0.23	3	0.32	0.53	5	0.36	0.69
Tt5	6	0.70	0.69	6	0.68	0.62	7	0.68	0.83	7	0.64	0.80
Tt8	13	0.96	0.89	13	0.91	0.89	9	0.86	0.83	9	0.82	0.86
Tt10	10	0.92	0.87	13	0.61	0.89*	14	0.43	0.72***	14	0.98	0.95
Tt17	6	0.61	0.79	6	0.64	0.69	6	0.50	0.58	3	0.55	0.62
Tt30	13	0.83	0.87	12	0.87	0.91	10	0.86	0.83	8	0.64	0.86*
Tt33	11	0.79	0.84	9	0.78	0.81	12	0.89	0.88	11	0.91	0.93
Tt34	3	0.50	0.55	4	0.52	0.51	5	0.18	0.26	4	0.55	0.72*
Tt37	14	0.75	0.91	13	0.91	0.87	10	0.68	0.68	9	0.99	0.89

APPENDIX D. CALIBRATION OF THE TELEMETRY ARRAY



a) Diagram illustrating the orientation of the transects conducted to estimate the variation of signal strength relative to distance from the telemetry antenna array. b) Radio tag attached to a clay model simulating a bat.